A therapeutic trial for safety and preliminary efficacy of the combination of axitinib and selenium in the chemical composition of selenomethionine (SLM) for adult patients with advanced metastatic clear cell renal cell carcinoma (CCRCC)

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IND number: **126767**

Study drug: **Selenomethionine (SLM)**

Phase: **1**

Indication: **Advanced metastatic renal cell carcinoma (Stage IV)**

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IRB # 201507716

SCHEMA

A Phase 1 trial for safety and preliminary efficacy of the combination of axitinib and selenomethionine (SLM) for adult patients with advanced metastatic clear cell renal cell carcinoma (CCRCC)

Escalation Part 1: COMPLETED

Dose-escalation: SLM administrated orally twice daily for 14 days, followed by once daily dosing in combination with axitinib 5 mg twice daily with titration according to package insert (listed in this protocol 5.1 Experimental Design Synopsis) in patients with advanced renal cell carcinoma. Treatment will continue until disease progression or unacceptable toxicity.

Expansion Part 2: ENROLLMENT COMPLETE

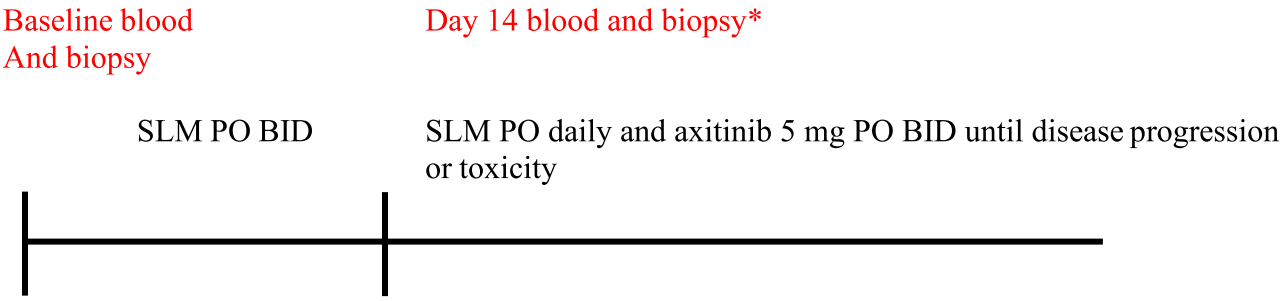
In this phase, patients will be treated at the maximum tolerated dose (MTD) of SLM determined in the Escalation Part 1 given orally twice daily for 14 days, followed by once daily dosing in combination with axitinib 5 mg twice daily with titration according to package insert (listed in this protocol 5.1 Experimental Design Synopsis) in patients with advanced renal cell carcinoma. Treatment will continue until disease progression or unacceptable toxicity.

Pilot phase: Extra 10 patients will be treated with SLM doses based on BSA as illustrated in the statistical part.

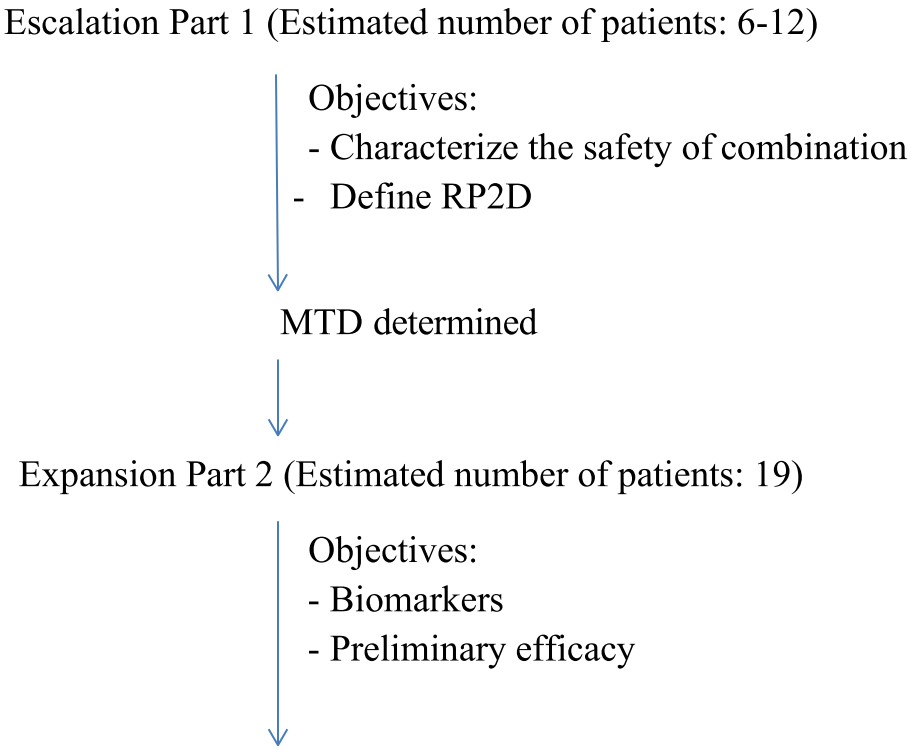
Patient population:

Advanced metastatic renal cell carcinoma (Stage IV), who have had at least one standard treatment or clinical trial including tyrosine kinase inhibitors (TKI), mammalian target of rapamycin inhibitors (mTOR-I) or immunotherapy

Experimental Design Schema showing the schedule for every patient enrolled in the study:



*The biopsy will be done only in the Expansion and Pilot in up to 20 carefully selected patients as detailed in the protocol, however the blood draw will be done on all patients.



Mandatory biopsy to be done on Day 1, 14 and Cycle 3 (8 weeks after first dose of SLM plus Axitinib) in expansion part 2 and pilot Phase , before and after giving SLM.

Refer to SLM Study Lab Calendar section 20.0 for SLM concentration blood draws.

Throughout the protocol SLM dose refers to the actual elemental selenium dose that patient is getting.

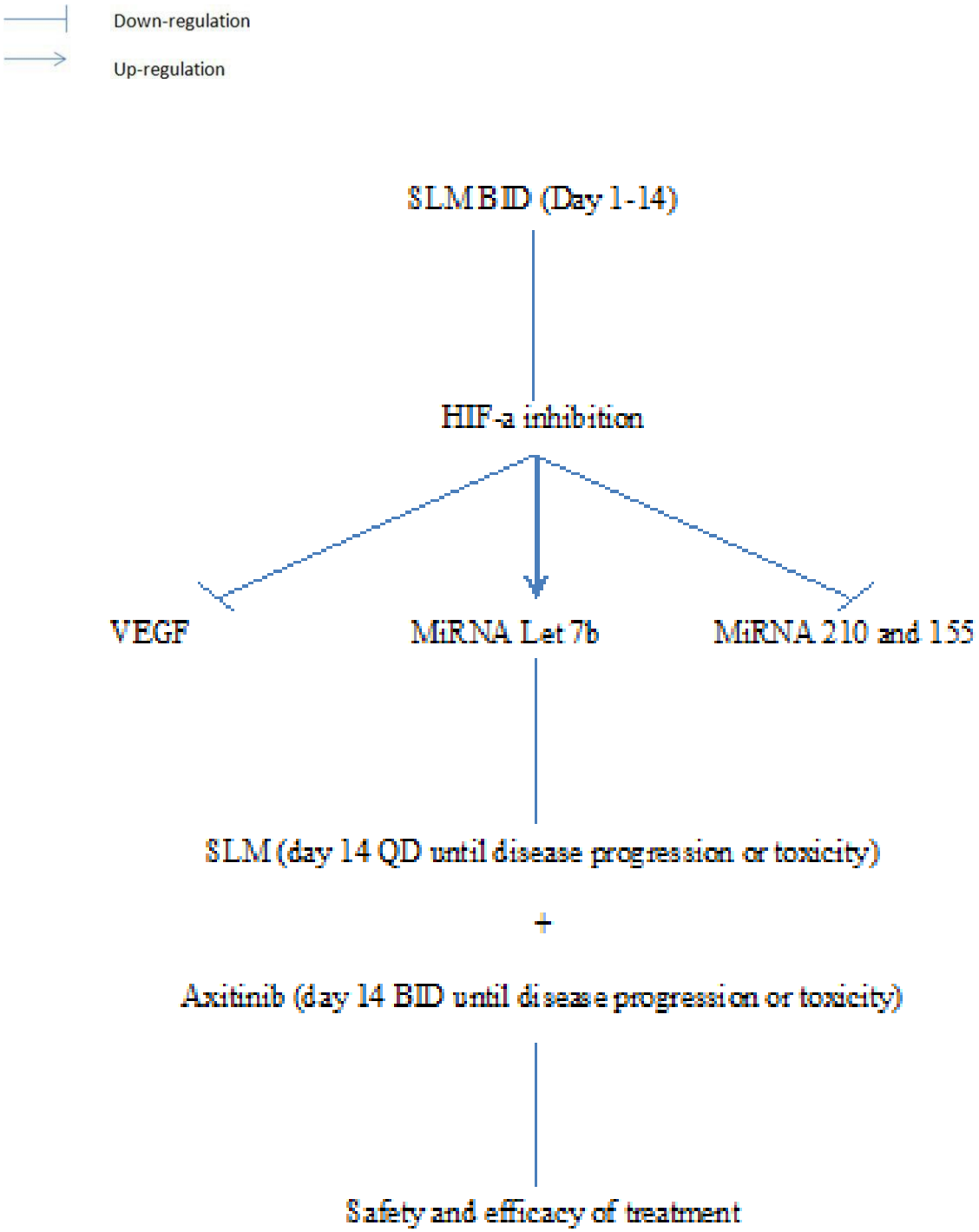

Down-regulation
Up-regulation
SLM BID (Day 1-14)
HIF-a inhibition
VEGF
MiRNA Let 7b
MiRNA 210 and 155
SLM (day 14 QD until disease progression or toxicity)
+
Axitinib (day 14 BID until disease progression or toxicity)
Safety and efficacy of treatment

Table of Contents

List of Abbreviations

AE	Adverse Event
ALT	Alanine Transaminase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
ATP	According to Protocol
BSA	Body Surface Area
CBC	Complete Blood Count
CCRCC	Clear Cell Renal Cell Carcinoma
CFR	Code of Federal Regulations
CR	Complete Response
CRSO	Clinical Research Safety Officer
CT	Computerized Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CYP	Cytochrome P450
DLT	Dose Limiting Toxicity
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDC	Electronic Data Capture
GCP	Good Clinical Practice
GPx	Glutathione peroxidase
HIF	Hypoxia-Inducible Factor
ICH	International Conference on Harmonization
INR	International Normalized Ratio
IRB	Institutional Review Board
ITT	Intent to Treat
LDH	Lactate Dehydrogenase
miRNA	micro RNA (Ribonucleic Acid)
MRI	Magnetic Resonance Imaging
MSA	Methylselenenic acid
MSC	Methylselenocysteine
MTD	Maximum Tolerated Dose
mTOR	Mammalian Target of Rapamycin

PD	Progressive Disease
PDGF	Platelet-Derived Growth Factor
PFS	Progression Free Survival
PHD	Prolyl Hydroxylase
PR	Partial Response
PSA	Prostate Specific Antigen
PTT	Partial Thromboplastin Time
RECIST	Response Evaluation Criteria In Solid Tumors
RP2D	Recommended Phase 2 Dose
RPCI	Roswell Park Cancer Institute
SAE	Serious Adverse Event
SD	Stable Disease
shRNA	short hairpin RNA
SLM	Selenomethionine
SPEER	Specific Protocol Exceptions to Expedited Reporting
TKI	Tyrosine Kinase Inhibitors
TMA	Tissue Microarray
TrxR	Thioredoxin reductase
VEGF	Vascular Endothelial Growth Factor
VHL	von Hippel-Lindau

1.0 OBJECTIVES

1.1 PRIMARY OBJECTIVES

1. To characterize the safety profile of combining SLM and axitinib in advanced metastatic CCRCC and;
2. To define the dose of SLM in combination with a standard dose of axitinib for Expansion Part 2 and future phase 2 trial (RP2D).
3. For the added pilot phase: The primary objective of this pilot dose-finding study is to characterize the dose-concentration relationship and estimate the effective administered dose of selenium necessary to achieve the target blood concentration range informed by preclinical data.

1.2 SECONDARY OBJECTIVES:

4. To determine that the dose of SLM administered is sufficient to alter the expression levels of HIF1a, HIF2a, VEGF and their associated micro-RNAs 210, 155, and LET7b.
5. To determine the SLM concentration when combined with axitinib.
6. To estimate the preliminary efficacy parameters of the combination of SLM and axitinib in terms of:
    1. Progression free survival (PFS),
    2. Objective response rate (SD+PR+CR), and
    3. Overall survival in patients with advanced metastatic CCRCC.

2.0 BACKGROUND

2.1 STUDY DISEASE (RENAL CELL CARCINOMA):

Clear cell renal cell carcinoma (CCRCC) is the most common type of kidney cancer and has high levels of hypoxia-inducible factor 1 alpha (HIF-1α) and hypoxia-inducible factor 2 alpha (HIF-2α) in addition to frequently mutated von Hippel-Lindau (VHL) gene. Majority of CCRCC are associated with VHL mutation/inactivation. Inactivation of VHL, which is a tumor suppressor gene, leads to the accumulation of HIF which in turn leads to the transcription of a variety of hypoxia responsive genes including VEGF, PDGF, erythropoietin etc and result in a highly vascular tumor (Escudier, Sternberg). Six novel treatments have been approved for use in metastatic/advanced kidney cancer in the past 5 years. The median progression free survival from the anti-angiogenic agents ranges from 5 months to 11 months. The anti-angiogenic therapies approved are sunitinib, sorafenib, and pazopanib all of which are tyrosine kinase inhibitors and the combination of bevacizumab (monoclonal antibody against VEGF-A) plus interferon. Other drugs, namely temsirolimus and everolimus, belong to the class of inhibitors of mammalian target of rapamycin (mTOR inhibitors) (Escudier, Sternberg). Resistance formation is an inevitable phenomenon and durable responses are rare with the above agents. HIF-α acumulation, proliferation of other growth factors and the so-called “angiogenic escape” are thought to play a significant role in the resistance formation (Casanovas).

It has been demonstrated using tissue microarray (TMA) that 92% (81 out of 88) of human primary CCRCC surgical specimens express HIF-α, using immuno-histochemical method developed by investigators at Roswell Park Cancer Center (RPCC). It is known that inactivation of prolyl hydroxylases (PHDs) lead to HIF-α stability, which in turn leads to trans-activation of several genes responsible for resistance formation to chemo and radiotherapies. HIF-α is hydroxylated by PHDs and degraded by the VHL-dependent ubiquitination via proteosomes. Renal cell carcinoma is treated by using signal transduction inhibitors that target the VEGF and mTOR pathway. Although HIF-a inhibition leads to tumor growth inhibition, the focus of the proposed research is to evaluate the effect of HIF-α inhibition by selenomethionine (SLM) alone in the pre-nephrectomy setting of CCRCC. Rustum et al have demonstrated that Se-Methylselenocysteine (MSC) decreases HIF-1α in head and neck cancer which in turn results in enhancing the efficacy of irinotecan, and showed that methylselenenic acid (MSA), the active metabolite of MSC inhibit both HIF-1α and HIF-2α in CCRCC cell lines namely RC2 and 786-0. As the above 2 cell lines have non-functional VHL, the results indicate VHL independent degradation of HIF-α by MSA. These findings provided the rationale to develop a new combination therapeutic strategy for CCRCC. This pilot study will evaluate the utility of SLM alone in HIF-α down-regualtion and regulation of down-stream markers including VEGF and miRNAs.

2.2 STUDY DRUG

Selenium (Se) is a natural element present in the earth’s crust often in association with sulfur-containing compounds. Humans get their dietary requirements mainly from food. Dietary Se is in the range of 29–152 μg/day in Europe and North America. Se is an essential trace element that is necessary for functioning of the enzyme glutathione peroxidase (GPx) and thioredoxin reductase (TrxR). As selenocysteine, it is a constituent of over 25 selenoproteins in the mammalian system. Maximum expressions of these selenoproteins require a daily dietary intake of 0.1–0.2 mg/kg in

animals and ~ 55 µg for humans (Jackson). Deficiency symptoms occur such as in Keshan disease when the consumption is < 20 µg/day. The anticarcinogenic properties of Se was noticed as a result of the inverse association between cancer mortality and crop Se contents (Shamberger) which was subsequently supported by findings of an inverse relationship between blood Se levels and prevalence of several types of cancer (Jackson). Se impacts multiple anticancer pathways that hinder cancer initiation, growth and progression. Some of these are summarized below:

1. Antiangiogenic and tumor vascular normalization effects leading to higher intratumoral drug delivery of anti-cancer drug/s used in combination chemotherapy (Bhattacharya)
2. Altered methionine metabolism leading to an increased sensitivity to caspase mediated apoptosis;
3. Retarding cell doubling time by increasing cell duration in G1, S and G2 phases of the cell cycle (Kaeck, Sinha)
4. Induction of genes involved in phase II enzyme activity and down-regulation of genes related to phase I enzyme and cell proliferation (Xiao H)
5. Inhibiting key targets such as cyclin A, cyclin D1, CDC25A, CDK4, PCNA, E2F gene expression and arresting cells in G1 phase of cell cycle (El-Bayoumy K)
6. Inducing expression of P19, P21, P53, GST, SOD, NQO1, GADD153 and certain caspases (El-Bayoumy K)
7. Possibly retarding metastasis since genes such as osteopontin is down-regulated by Se (El-Bayoumy K) and,
8. Modulating the immune system (combs).

2.3 CLINICAL STUDIES WITH SELENOMETHIONINE

Se compounds have been used in various clinical trials, mainly in chemo-prevention, at doses in the nutritional range (50 µg/day to 200 µg/day) without any toxicity (Casanovas). Since SLM was evaluated at higher doses 10 mg/kg/d in preclinical models where therapeutic synergy with anticancer drugs was demonstrated, clinical trials with escalating doses of SLM to achieve plasma concentration determined optimal in preclinical models were initiated at RPCI by Dr. Fakih, et al (Fakih) in combination with fixed dose of Irinotecan 125 mg/m2 weekly. Different dose levels of SLM have been used (3200, 4000, 4800, 5600, and 7200 µg PO BID loading dose followed by once daily maintenance dose). A dose of 7200 µg/BID x 7 days followed by 7200 µg/QD for up to 36 days was determined sufficient and without toxicity. The only toxicity attributed to SLM was mild garlic-like odor (breath and urine) and was limited to G1 in about 50% of patients. This was seen more commonly during the induction SLM week and tended to ameliorate or disappear with prolonged treatment. No skin or nail toxicities secondary to SLM were documented.

Preliminary data from the initial trial indicate a single fixed dose of selenium conferred significant variability in patient blood selenium concentrations at the end of the 14-day run-in. Patient body surface area (BSA) was found to be a contributing factor with patients of lower BSAs more frequently having higher blood selenium concentration, although none of which were within the targeted range of 35-45 µM. Here we list the current subjects in 4 cohorts (C1: 4000 mcg, C2: 2500 mcg, C3: 3000 mcg and C4: 4000 mcg) and we list blood selenium concentration at D14, Cycle 2 and 7. There is clear difference in C 4 and C1 average blood concentration being higher in C 1 and associated with better responses.

Cohort	Pre	D14	C2	C7	Response
1	2.1	30.0	44.0	104.0	2CR, 1PR
2	2.0	14.0	21.0	25.0.	1PR, 1 PD
3	1.8	17.0.	25.0	43.0.	1PR, 2 PD
4	2.2	17.5	27	64	2 SD, 1 PD

CR (COMPLETE RESPONSE), PR (PARTIAL RESPONSE) SD (STABLE DISEASE) PD (PROGRESSIVE DISEASE)

IN LIGHT OF THESE FINDINGS, A MODIFIED DOSING SCHEME IS BEING INVESTIGATED WHICH VARIES THE DOSAGE OF SELENIUM BY BSA WITHIN A DOSE LEVEL TO ENSURE LESS VARIABILITY IN THE DOSE PER M2 ADMINISTERED.

2.4 HYPOXIA INDUCED FACTOR (HIF-A):

Many solid cancers contain regions of hypoxia. Rapid cancer cell proliferation is faster than the proliferation of the endothelial cells forming very irregular and chaotic enovasculature, which results in the development of regional hypoxia in the tumor. Intratumoral hypoxia activates the key transcriptional factor, hypoxia – inducible factor 1 (HIF-1α). This mediates the activation of more than one hundred genes in tumor cells to adapt to a low oxygen environment, and promote continued tumor growth, resistance to chemo/radiotherapy. HIF-1α is expressed in a wide range of human solid tumors and its expression correlates with increased angiogenesis, chemo/radio resistance, and poor patient prognosis. Current efforts are underway to develop HIF-1α inhibitors, and to test their efficacy as potential anticancer agents. (Semenza, Mabjeesh, Hirota)
As with other proteins, the level of HIF-1α cellular accumulation is determined by the rate of protein synthesis and degradation. Under normoxic conditions, oxygen dependent hydroxylation of prolin in HIF-1α by two enzymes, prolyl hydroxylase 2 and 3 (PHD2, 3), is the key step which leads to the recognition of HIF-1α by von Hippel – Lindau (VHL) protein, and degradation through the ubiquitin-proteosome pathway (Figure 1). Therefore, under normoxic conditions, like in normal organs, HIF-1α is rapidly degraded and thus undetectable. Under hypoxic conditions, however, proline hydroxylation and the level of PHD2 and 3 decreases, and VHL cannot bind to HIF-1α, resulting in a decreased rate of HIF-1α degradation, thus HIF-1α is expressed under hypoxia. (Semenza, Hirota)

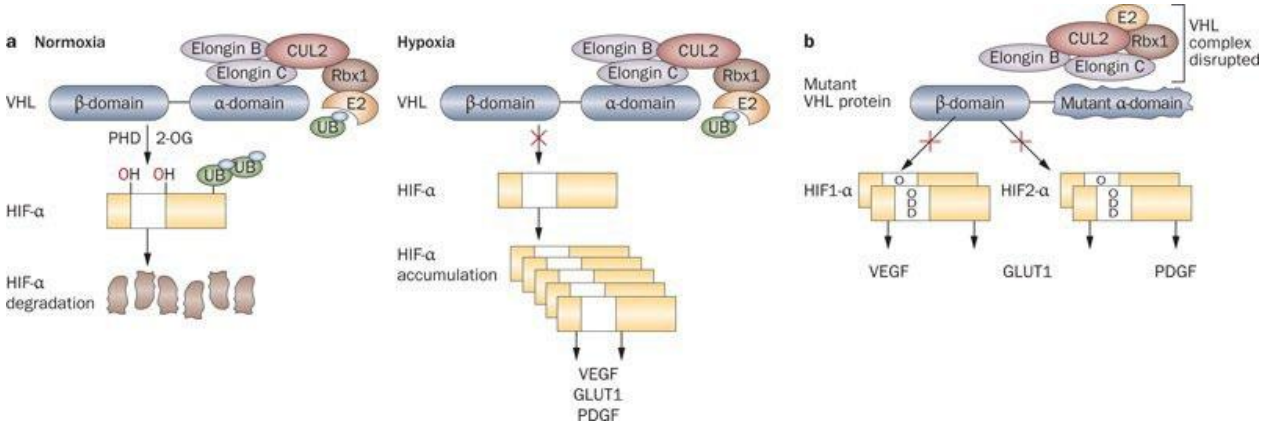


Figure 1: Linehan, W. M. *et al. Nat. Rev. Urol* doi:10.1038/nrurol.2010.47

2.5 AXITINIB AS A SECOND LINE THERAPY IN METASTATIC CCRCC

Axitinib is an oral, potent, and selective inhibitor of VEGF receptors 1, 2, and 3. In a phase II study, axitinib was found active in cytokine-refractory metastatic renal cell cancer (Rixe). Responses were reported with the use of chemotherapy, hormone or biological treatment. The objective response rate was 44·2% for patients treated with axitinib. In a phase III trial comparing axitinib to sorafenib as second line treatment for advanced CCRCC, PFS was 6.7 months in the axitinib arm and 4.7 months in the sorafenib arm ($p<0.0001$), with manageable toxicities (Rini, lancet). Thus CCRCC offers an excellent opportunity for proof of concept, in a relatively small sample size, that sustained inhibition of HIF-α and VEGF by the sequential and concurrent combination of SLM and axitinib is safe and could possibly impact significantly on treatment outcome.

Approach to treatment of clear cell renal cell carcinoma

Setting		First choice	Alternative
First-line therapy	0 to ≤2 risk factors	HD IL-2	Sunitinib
			Bevacizumab plus IFN-alfa
			Pazopanib
	>2 risk factors	Temsirolimus	Sunitinib
Second-line therapy	Prior cytokine	Axitinib	Pazopanib
			Sunitinib
		Sorafenib	Temsirolimus
			Bevacizumab
	Prior VEGFR inhibitor	Everolimus	Clinical trials
		Axitinib	
		Sorafenib	
	Prior mTOR inhibitor	Clinical trials	

Risk factors: poor Karnofsky Performance Status, serum lactic dehydrogenase (LDH) level >1.5 times the upper limit of normal, corrected serum calcium >10 mg/dL (2.5 mmol/L), hemoglobin concentration less than the lower limit of normal, and absence of nephrectomy (ie, no disease-free interval).

HD IL-2: high-dose interleukin 2; IFN-alfa: interferon alfa; mTOR: mammalian target of rapamycin; VEGFR: vascular endothelial growth factor receptors.

Courtesy of Dr. Michael Atkins.

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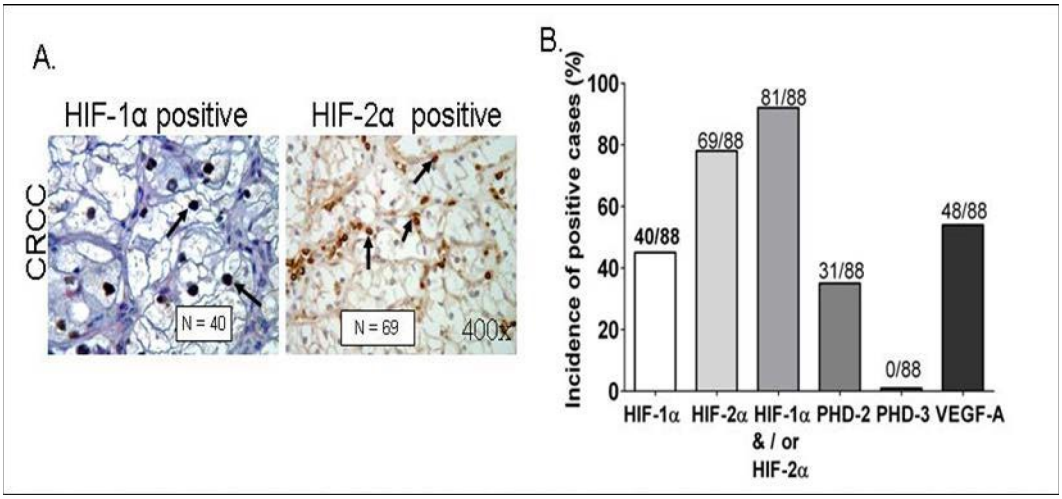
2.6 STUDY RATIONALE

2.6.1 Rationale for Targeting HIF-α and VEGF-A Clinical Data

The data in Figure 2 also demonstrate that while the level of VEGF-A in CCRCC tumor is relatively low in intensity and incidence (54%), the protein level of VEGF-A in colorectal and head and neck cancer is 97% and 75%, respectively. Thus, with the documented molecular profile of CCRCC and the demonstrated ability of selenium to inhibit HIF-1α, HIF-2α, and VEGF-A and activate PHDs in preclinical models, it is critical to confirm whether selenium can effectively inhibit HIF-α and VEG in patients with CCRCC without toxicity as proof of principle. The exact mechanism is not known at this time, it is hoped that this will be studied in the future. Effective demonstrations of the effect of SLM in the clinic would provide the rationale and scientific basis to combine SLM with other agents in future Phase II trials to determine if such an approach is therapeutically sound.

In addition to assessing effects of SLM on HIF-α and VEGF-A protein expression, pretreatment with SLM, as anti-angiogenic agents, could offer clinical advantage, namely down-staging that could facilitate surgery and benefit from nephrectomy.

Figure 2. Immunohistochemical Staining of HIF-1α and HIF-2α in CCRCC Tumor and the Incidence of HIF-α and VEGF-A in CCRCC Tumors.



2.6.2 Clinical Safety of SLM using the Proposed Dose (3600µg/m^2 equivalent to 7200 µg, assuming patient BSA is 2.0m^2)

Clinical Phase I and II trials of escalating dose of SLM was conducted by Dr. Marwan Fakih at RPCI (Fakih). The data in Figure 4 is a summary of plasma concentration of selenium achieved at various doses of SLM administrated orally, BID. The desired plasma concentration of approximately 30 µm, a concentration formed to offer effective inhibition of HIF-α and VEGF and therapeutic synergy in preclinical models, was achieved without toxicity, except the garlic breath. Thus, the proposed highest dose 3600µg/m^2 to be administrated twice daily should be safe and hopefully effective in inhibiting HIF-α and VEGF in patients with CCRCC.

2.6.3 Preclinical Data in Support of the Rationale for the Proposed Trial

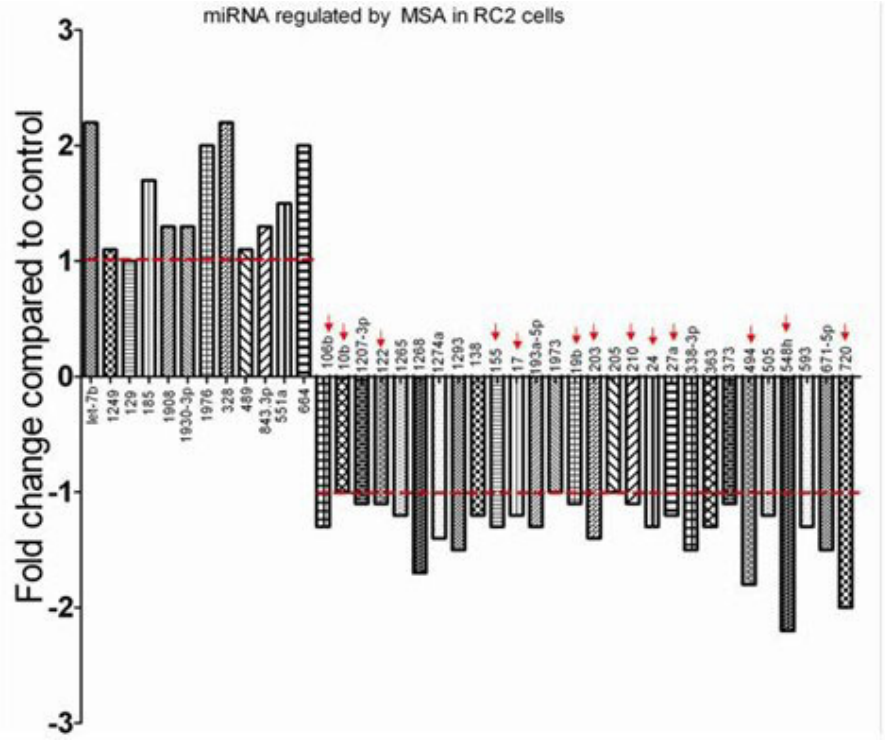
2.6.3.1 An Inverse Relationship between the Combined Expression of HIF-1α and HIF-2α and Prolyl Hydroxylase – 2 (PHD$_2$) In CCRCC Tumor Specimens

Data in **Figure 2** demonstrates that in 88 Tissue Microarray (TMA) of primary tumor biopsy specimens, the cumulative expression of HIF-1α and HIF-2α is 92% (81/88), with HIF-2α being the dominant type, 78% (69/88), markers associated with increase tumor angiogenesis and resistance to chemotherapy and radiation therapy. In contrast, the tumors express lower incidence and intensity of PHD$_2$, are deficient in PHD$_3$ and VHL (data not shown), and are molecular markers associated with hydroxylation of HIF-α and degradation in the proteasome. Unlike other solid tumors tested in Dr. Rustum's laboratory (colorectal and head and neck), CCRCC is unique in expressing high incidence and intensity of HIF-α, lower levels of PHD$_2$ and VHL, and in being PHD$_3$ deficient. It is

hypothesized that the stable expression of HIF-α in CCRCC is due to the low intensity and incidence of PHD_2 and PHD_3 and deficiency in VHL.

2.6.3.2 Using microarray analysis, MSA treatment resulted in the downregulation of 27 miRNA and the upregulation of 12 miRNA. Several miRNA were significantly modulated by MSA. Analysis of four metastatic biopsies revealed the upregulation of miRNA 210 and 155 and the downregulation of Let-7b and 328 miRNA. Thus, further analysis for a possible relationship between HIF-α expression and specific miRNA expression should be documented.

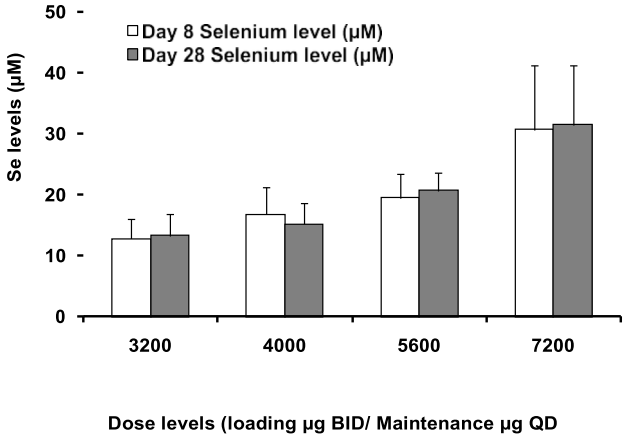
Figure 3. miRNA Profile of RC_2 Cells Treated with MSA (Chintala, Rustum, unpublished and confidential)



2.6.3.3 Plasma Levels of Selenium in Patients Treated with Escalating Doses Of SLM

The data in Figure 4 is the tabulation of Selenium levels achieved following the oral administration to colorectal cancer patients of SLM up to 7200 µg x 7d. The data generated in collaboration with Dr. Fakih (Fakih) demonstrated that the derived plasma level can be achieved with 7200 µg BID without major toxicity. This dose of 7200 µg BID is equivalent to the proposed dose for this study of 3600 µg/m2 (7200 µg BID assuming patient's body surface area is approximately 2 m^2), which could produce plasma SLM levels of ≥ 30 µM.

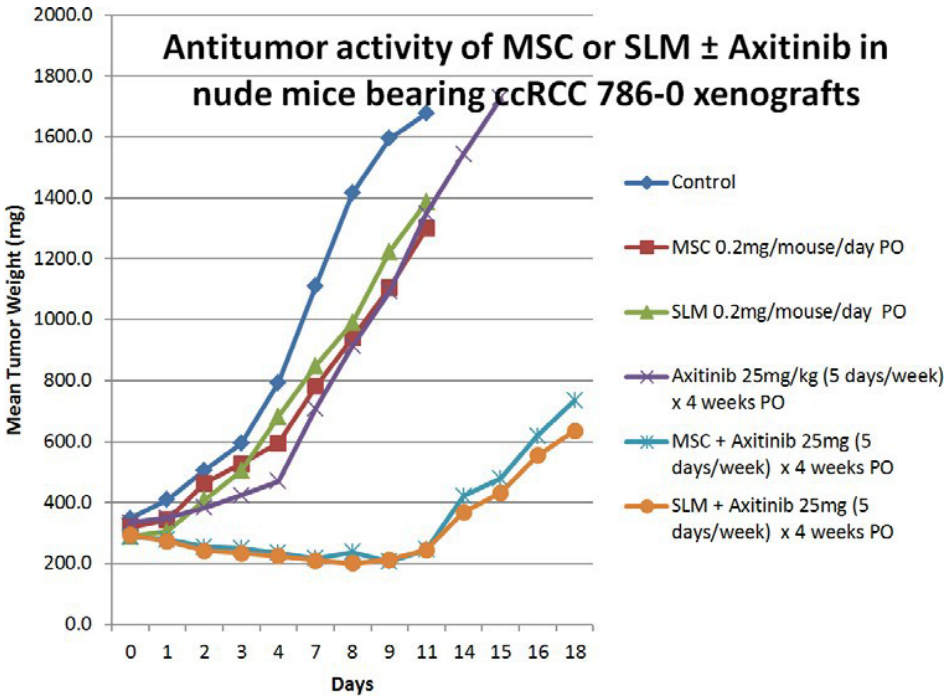
Figure 4. Se Level on Day 8 and Day 28 in Patients Receiving SLM – Plasma Levels of SLM in Patients Treated with Escalating Dose of SLM (12, 13)



2.6.3.4 Selenium Pretreatment Enhances the Antitumor Activity and Prevents the Toxicity Associated with Chemotherapy (Cao, Chintala, and Rustum unpublished data)

The data in Figure 5 demonstrates that nontoxic doses of selenium can effectively enhance therapeutic efficacy of Axitinib in human tumor CCRCC 786-0 xenografts. In fact, tumors that are resistant to drugs can be sensitized by selenium treatment. In previous published results, selenium was effective in protecting normal tissues from toxicity induced by a number of chemotherapeutic agents (Vaughan). These preclinical results clearly demonstrated the therapeutic potential of combining selenium with Axitinib.

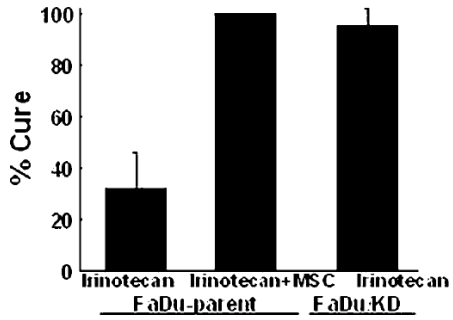
Figure 5. MSC or SLM Augments the Antitumor Activity of Axitinib in Nude Mice Bearing Human Tumor Xenografts



2.6.3.5 Inhibition of HIF-α Correlate with Enhanced Antitumor Activity

Data in Figure 6 indicates that inhibition of HIF-α by selenium or by shRNA knockdown resulted in similar, but high cures of xenografts bearing head and neck tumors. These data supported the hypothesis that inhibition of HIF-α is critical for therapeutic synergy in vivo.

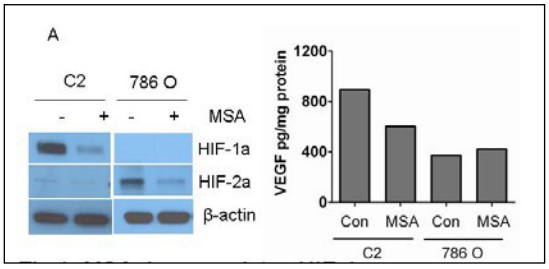
Figure 6. Inhibition of HIF-α Is Critical for Therapeutic Efficacy



2.6.3.6 Selenium Downregulates the Protein Expression of HIF-1α, HIF-2α, and VEGF in CCRCC Tumor Cells (Chintala S)

The data in Figure 7 indicate that selenium can effectively inhibits both HIF-α in CCRCC and such inhibition correlated with down regulation of VEGF, as transcriptionally regulated by HIF-α. Thus, inhibition of HIF-α by selenium is not tumor specific, inhibits HIF-α in colon, head and neck, and CCRCC. Further, more inhibitions of HIF-α is independent of VHL status. While colon and head and neck tumors express functional VHL, CCRCC tumor cells are deficient of VHL (Linehan). The finding that HIF-α inhibition is VHL independent but PHD dependent, provided strong evidence that selenium, unlike other HIF-α inhibitors, is unique in that inhibition of HIF-α is achieved via enhanced degradation by PHDs, a unique and new mechanism.

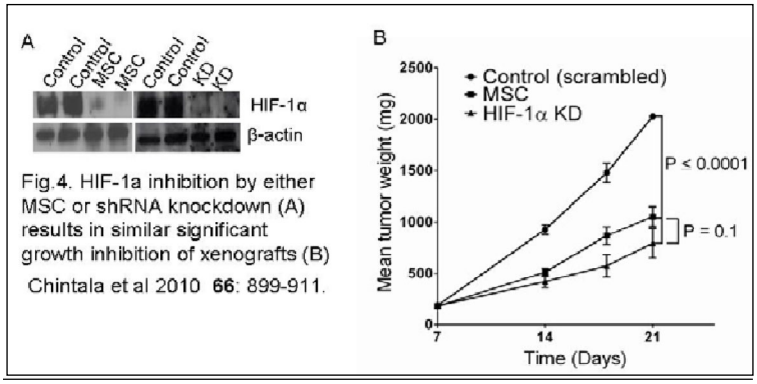
Figure 7. MSA Downregulates HIF1-α and HIF-2α and Decreases VEGF secretion



2.6.3.7 HIF-α Inhibition by SLM or Stable Knockdown Significantly Inhibited the Growth of Tumor Xenografts

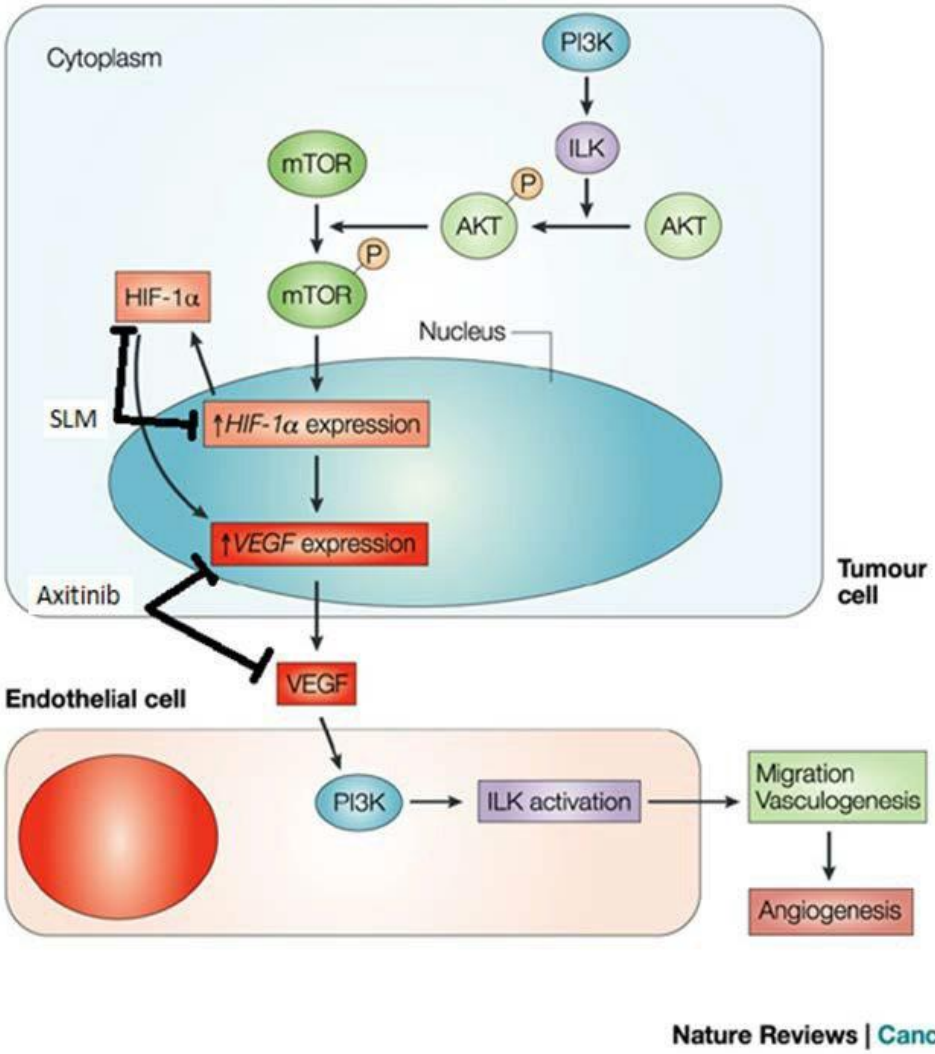
It has been demonstrated that HIF-1α inhibition by SLM in FaDu tumor xenografts lead to a significant, 30% growth inhibition. Furthermore Rustum et al have confirmed this growth inhibition by knocking down HIF-1α using stable HIF-1α shRNA, demonstrating that preclinical xenografts model (**Figure 8**).

Figure 8. HIF-1α Inhibition by Either MSC or shRNA Knockdown Results in Similar Growth Inhibition of Xenografts



2.7 HYPOTHESIS TO BE TESTED:

Based on therapeutic and mechanistic data generated in Dr. Rustum's laboratory, and on the demonstrated clinical safety of the proposed SLM dose, the hypothesis to be tested in this laboratory-based clinical trial is that therapeutic, nontoxic doses of SLM will down-regulate the high expression levels of HIF-a, VEGF and its associated miRNAs, and such an effect will sensitize tumor cells to treatment with axitinib, a TKI inhibitor, resulting in enhanced treatment outcome and potentially reversal of resistance to TKI inhibitors.


Cytoplasm
PI3K
ILK
mTOR
P
AKT
AKT
P
mTOR
HIF-1α
Nucleus
SLM
↑HIF-1α expression
↑VEGF expression
Axitinib
Tumour cell
VEGF
Endothelial cell
PI3K
ILK activation
Migration
Vasculogenesis
Angiogenesis
Nature Reviews | Cancer

3.0 PATIENT SELECTION

3.1 ELIGIBILITY CRITERIA

Each patient must meet all of the following criteria to be enrolled in this study:

1. Histologically and radiologically confirmed advanced metastatic CCRCC in patients who have had at least one prior systemic therapy, which can include axitinib for the dose escalation part. In the expansion and pilot phases, patients with prior axitinib are allowed, as long as the last dose of axitinib was longer than 6 months ago.
2. Written and voluntary informed consent.
3. At least one Response Evaluation Criteria In Solid Tumors (RECIST)-defined target lesion. *Patient must have documented disease progression.
4. Renal function (creatinine level within normal institutional limit, or creatinine clearance >15 mL/min/1.73 m2 for patients with creatinine levels above institutional normal, calculated using the Cockcroft-Gault formula).
5. Liver function (AST/ALT ≤2.5 X institutional upper limit of normal **OR** ≤ 5 x institutional upper limit of normal in cases of liver metastases; Total bilirubin ≤ 1.5 times ULN.)
6. Adequate hematological lab values including:
   - Absolute Neutrophil Count (ANC) ≥ 1.0 x 10^9/L
   - Platelets ≥ 100 x 10^9/L
   - Hemoglobin ≥ 7.0 g/dL
7. Eastern Cooperative Oncology Group (ECOG) performance status of 0 (fully active, able to carry on all pre-disease performance without restriction), 1 (restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, such as light housework or office work) or 2 (Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours).
8. Age of at least 18 years.
9. Life expectancy of 12 weeks and more.
10. 2 weeks or more since end of previous systemic treatment (4 weeks or more for bevacizumab plus interferon-alfa). 3 days wash out for palliative radiation.
11. Must have a safely accessible biopsy per treating physician and the provider performing that biopsy. Patient must agree to have this biopsy done as outlined in the calendar. If patient does not have safely accessible biopsy, the patient may still be enrolled per investigator discretion.

3.2 EXCLUSION CRITERIA:

Patients eligible for this study must not meet any of the following criteria:

1. Patients with prior malignancies of the same or different tumor type in the last 5 years and patients with

concurrent malignancies of the same or different tumor type UNLESS the natural history of the disease or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational drug.

2. Symptomatic untreated metastases in the central nervous system.
3. Subject that is pregnant or lactating.
4. Pre-existing uncontrolled hypertension defined as > 150/90 mm Hg with medication.
5. Present use or anticipated need for cytochrome P450 (CYP) 3A4-inhibiting, CYP3A4-inducing drugs (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, and voriconazole, rifampin, phenytoin, carbamazepine, rifabutin, rifapentin, phenobarbital, and St. John's wort, bosentan, efavirenz, etravirine, modafinil, and nafcillin).
6. Myocardial infarction, uncontrolled angina, congestive heart failure, or cerebrovascular accident within previous 6 months. Subjects with history of deep vein thrombosis or pulmonary embolism, at provider discretion.
7. Major surgery within 4 weeks of starting study treatment.
8. Known HIV or acquired immunodeficiency syndrome-related disease.

3.3 INCLUSION OF WOMEN AND MINORITIES

- No exclusion is made on the basis of sex, race or ethnic background.

4.0 ENROLLMENT PROCEDURES

4.1 ENROLLMENT PROCESS

All patients must be enrolled on study before beginning therapy.

4.1.1 Consent Process

Subjects will be consented by a member of the study team after receiving an explanation of the study, review of the consent form, and having had all questions answered by a member of the study team. . The subject will be provided with a copy of the signed consent form.

An automatic notification will be sent to appropriate staff, including the safety officer after consent, following entry of patient information into the clinical trials management system (OnCore).

4.1.2 Eligibility Assessment:

Eligibility will be assessed according to the protocol. An eligibility check list will be completed and signed by the treating physician, documenting his/her review of all eligibility criteria and confirming all screening assessments have been completed and the subject meets criteria to begin protocol treatment. Once eligibility is confirmed by the treating physician, a signed copy of the eligibility checklist will be scanned and entered into OnCore and automatic notifications sent to appropriate staff.

Disease assessment is to be performed within the 28 days prior to the start of study treatment using CT scan of chest, abdomen and pelvis with or without bone scan based on provider's judgment. Patient disease assessment will be performed according to RECIST 1.1 criteria for solid tumors.

The following pre-treatment evaluations are to be performed within 28 days prior to the start of treatment:

- Compliance with eligibility criteria
- Patient demography
- Medical history (to include a complete history of previous, present and concomitant conditions and treatment)
- Physical examination (complete physical examination, including height, weight and BSA)
- Vital signs (to include blood pressure, heart rate, respiratory rate, and body temperature)
- Assessment of ECOG performance status
- CBC with differential to include: hemoglobin, differential white blood cell count, platelet count. Coagulation parameters including INR (International Normalized Ratio), aPTT, and PT.

- Clinical chemistries to include: ALT, AST, total bilirubin, sodium, potassium, calcium, magnesium, urea, creatinine (creatinine clearance calculated using the Cockcroft and Gault formula), total protein, albumin, LDH, Alkaline phosphate.
- Urinalysis
- Glucose
- Erythropoietin per calendar
- Assessment of cardiac risk factors (Lipid profile, smoking, family history of cardiac disease, use of aspirin)
- Concomitant medications
- 12-Lead ECG after 5 minutes supine rest.
- Pregnancy test (this test is mandatory in women of child-bearing potential)
- Dual Energy CT Scan – Expansion and pilot Phases Only

5.0 TREATMENT PLAN

5.1 EXPERIMENTAL DESIGN SYNOPSIS

The study is designed as a prospective Phase 1 trial of SLM and axitinib in adult patients with advanced or metastatic clear cell renal cell carcinoma, who have had at least one standard treatment including tyrosine kinase inhibitors (TKI) or mammalian target of rapamycin inhibitors (mTOR-I). There must be imaging confirmation of tumor progression after completing a course of standard treatment. SLM and Axitinib will be provided through the study. The information below regarding the dose levels is not applicable to the Pilot phase.

Dose Levels fo Escalation and Expansion Phases	**Selenium in the chemical composition of SLM**	**Axitinib**
-2	1500 µg administrated orally twice daily for 14 days followed by 1500 µg once daily in combination with axitinib	5 mg oral twice daily with titration according to package insert (listed in this protocol 5.1 Experimental Design Synopsis)
-1	2500 µg administrated orally twice daily for 14 days followed by 2500 µg once daily in combination with axitinib	5 mg oral twice daily with titration according to package insert (listed in this protocol 5.1 Experimental Design Synopsis)
0	3000 µg administrated orally twice daily for 14 days followed by 3000 µg once daily in combination with axitinib	5 mg oral twice daily with titration according to package insert (listed in this protocol 5.1 Experimental Design Synopsis)
1 (starting dose)	4000 µg administrated orally twice daily for 14 days followed by 4000 µg once daily in combination with axitinib	5 mg oral twice daily with titration according to package insert (listed in this protocol 5.1 Experimental Design Synopsis)

****All dose levels: starting on day 15, SLM will be taken once daily in combination with axitinib**.

Escalation Part 1: COMPLETED: Last patient enrolled 10/23/2018.

Patient will start the assigned dose of SLM for 14 days and then will resume taking SLM orally at the same dose, combined with axitinib 5 mg twice daily with titration according to package insert (listed in this protocol 5.1 Experimental Design Synopsis).

Safety assessment will follow the guidelines provided in the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) Version.4.03.
Patients will be followed both clinically and radiographically (CT) at baseline then every 8 weeks after starting the treatment. Responses will be assessed using RECIST 1.1 criteria.
Dose level 1 (4000 µg) will be the starting dose in the standard 3+3 dose escalation design. Three patients will be recruited for Dose level 1.

The next three patients will be recruited for Dose level -1 (2500 µg). While none of the three patients in Dose level 1 experienced a DLT, there were significant side effects.
If 0 of three patients at Dose level -1 experiences a DLT related to SLM, then dose escalation will proceed to the next dose level (3000 µg).

If one of the three patients at Dose level -1 experiences a DLT related to SLM, then three more patients will be treated at the same dose. If exactly one out of six patients experiences a DLT, then dose escalation will proceed to the next dose level (3000 µg) after discussion at the weekly SLM meeting.

If two of the first three patients experience DLT related to SLM, then the dose will de-escalate to the lower dose (1500 µg).

The next patient can be enrolled after the previous patient's 28th Day of SLM assessment, provided there are no DLTs related to SLM. The minimum number of patients required is 6 and the maximum is 12.

Dose Limiting Toxicities:
DLTs related to SLM will be defined as any of the following events determined by the Investigator to be related to treatment combination irrespective of outcome. This applies to the first cycle only (2 weeks SLM alone + 4 weeks combination therapy):

- Clinically significant non-hematologic toxicity of Grade 3 or greater not related to underlying malignancy.
- Severe hematological toxicity (Grade 4 neutropenia, Grade 3-4 febrile neutropenia, or Grade 4 thrombocytopenia).
- If resolution of DLT related to SLM does not occur in 4 weeks, then patient will be withdrawn from the study.
- Grade 4 nausea, vomiting, diarrhea, or electrolyte imbalances or Grade 3 nausea, vomiting, diarrhea and electrolyte imbalances lasting greater than 48 hours despite optimal medical intervention.
- In the dose escalation phase, treatment delays related to the study drug that last >2 weeks

- Any grade 3 or higher DLT related to SLM alone will be discussed at the weekly SLM committee meeting.
-

Expansion Part 2: Enrollment Completed

After completing the Escalation Part 1 and based on the safety results, we will initiate an expanded cohort, Expansion Part 2 including a total of 19 patients with advanced metastatic CCRCC, to be treated with SLM 4000 in combination with axitinib 5 mg PO BID.

Up to 20 patients in the Expansion Part 2 and pilot phase will have mandatory biopsies at baseline before the start of SLM that will be repeated around day 14 (+/- 3 days) after starting SLM, at Cycle 3 (8 weeks +/- 3 days after first dose of SLM plus Axitinib) and preferably at disease progression, preferably from the same site of the previous biopsy, to study the expression of HIF-a and VEGF by immunohistochemistry (IHC), along with other biomarkers detailed in the correlative studies, before and after treatment with SLM. These tissues will be flash frozen and stored at -80 C to be processed later all at once for the proposed biomarkers.

The candidate patients for the biopsies will be chosen carefully by the treating oncologist and after discussion with the interventional/ body radiologist. Decision will be based on safety, ease of accessibility, tumor size and feasibility of getting the biopsy which can be done either on the primary renal tumor or metastatic site. Patient has to agree to get the biopsies, and sign consent form, after discussing the risks and benefits of the procedure with the provider.

All patients will have a peripheral blood draw at baseline before the start of SLM and repeated on day 14 after starting treatment. This blood will be stored and may be used later to study the protein expression of HIF1a, HIF2a, VEGF and level of SLM.
For Escalation Subjects: All patients will have a peripheral blood draw at baseline before the start of SLM and repeated on day 14 after starting treatment.
For Expansion Subjects: the peripheral blood draw will occur pre-dose and 2 hours post dosing on day 1 then 2 hours post dosing on Day 14 and at Cycles 2, 3, 4, 7, 10 and 13.

Pilot Phase: BSA based dosing. See additional details in protocol Section 14.4.

5.2 STUDY AGENT ADMINISTRATION

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 8. Appropriate dose modifications are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy. Treatment with SLM and axitinib will continue until disease progression or unacceptable toxicities or patient meets any criteria for removal from study as outlined in section 5.6.1 After completion of the SLM run-in period, each cycle will be 28 days in length.

5.3 SLM AND AXITINIB ADMINISTRATION

SLM oral capsules will be given twice daily alone for the first 14 days, then once daily in combination with Axitinib thereafter. SLM dosing will be based on patient BSA for the Pilot phase and tolerance based on DLT assessments during run-in only. See section 14.4 for dosing information for the Pilot portion. SLM will be provided by the study free of charge, by Sabinsa Corporation located in NJ, USA. SLM will be stored in the Investigational Pharmacy at University of Iowa Hospitals and Clinics.

Axitinib oral tablets will be provided by the study free of charge from Pfizer and stored in the Investigational Pharmacy at the University of Iowa Hospitals and Clinics. Dosing will start at 5 mg twice daily, with the two doses taken 12 hours apart, with or without food. Axitinib will begin after the patient completes the 14 day SLM run-in portion of the trial.

Over the course of treatment, patients who tolerate Axitinib for at least two consecutive weeks with no adverse reactions >Grade 2 (according to the Common Toxicity Criteria for Adverse Events [CTCAE]) may have their dose increased as follows.

When a dose increase from 5 mg twice daily is recommended, the Axitinib dose may be increased to 7 mg twice daily, and further to 10 mg twice daily using the same criteria.

5.4 GENERAL CONCOMITANT MEDICATION AND SUPPORTIVE CARE GUIDELINES

No premedication will be recommended. All standard of care antiemetics, antidiarrheals, or antibiotics are permitted if clinically indicated. During the course of treatment, if there is a need for palliative intervention such as radiation therapy or surgery, subjects are allowed to stay on study, and it will be to the discretion of the treating provider and study PI whether to continue or hold study combinations drug. Treating physician will take into consideration, safety and toxicity of the procedure and the patient's best interest.

5.5 DURATION OF FOLLOW-UP

Patients will be followed up, until they are lost to follow-up or death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed for resolution or stabilization of the adverse event as well. Follow up off study will be performed using telephone contact correspondence with treating physicians, and death records as necessary to update vital status at least every 6 months.

5.6 EVALUATIONS DURING SCREENING AND TREATMENT:

The following evaluations and assessments will be performed at the specified times listed in the study calendar (section 11.0):

- Physical examination
- Vital signs including blood pressure with each visit
- Assessment of ECOG performance status
- Clinical Chemistries
- CBC with differential
- Urinalysis
- Glucose
- Erythropoietin levels
- Lipid Panel
- Coagulation levels
- Blood sample(s) for SLM concentration analysis, miRNA samples and additional blood for storage.
- Pregnancy test for women of childbearing potential.
- EKG
- Dual Energy CT Scans will occur at Screening, Day 14 and Cycle 3(8 weeks after first dose of SLM plus Axitinib)
- Disease assessments CT +/- Bone Scan will begin at Cycle 5 Day 1(every 8 weeks +/- 5 days). These scans will be performed as determined by the treating physician. If subject is asymptomatic, bone scans may be done every 4 months.
- Concomitant medications
- All treatment-related adverse events should be followed until resolution or stabilization.
- Safety phone call to assess subject status.Archival tumor tissue collection, if availableFresh Tumor Tissue Biopsy at Screening, Day 14 and Cycle 3 (+/- 3 days both occasions) and preferable at progression, for up to 20 patients in Expansion Phase and the Pilot phase, if accessible and deemed safe by physician.

5.6.1 CRITERIA FOR REMOVAL FROM STUDY

Patients will continue study treatment until any of the following occur:

- Disease progression (treatment beyond progression at the discretion of treating provider with confirmatory scan in 8 weeks if the patient is clinically doing well).
- Unacceptable toxicity
- Intercurrent illness that prevents further safe administration of treatment
- Withdrawal of consent
- Serious protocol violation
- Non-compliance to therapy regimen
- General or specific changes in the patient's condition rendering the patient unacceptable for further treatment, in the judgment of the investigator

The reason for study removal and the date the patient was removed must be documented on the Case Report Form.

5.7 END OF TREATMENT:

The end-of-treatment visit is to be conducted 28 days (+/- 7 days) after treatment discontinuation, and will include the following assessments:

- Physical examination
- Vital signs including blood pressure
- Assessment of ECOG performance status
- Clinical Chemistries
- CBC with differential
- Glucose
- Urinalysis
- Assessment of adverse events
- Concomitant medications
- Mandatory Fresh Tumor Tissue Biopsy at disease progression, for up to 20 patients in Expansion and pilot Phase, if accessible and deemed safe by physician.

5.8 EVALUABLE POPULATIONS:

For Escalation Part 1: the DLT evaluable population includes all patients who complete one cycle of SLM and patients who experience a DLT during the first cycle of dosing.

For Escalation Part 1 and Expansion Part 2: The safety population includes all patients who receive at least one dose of SLM.

For Expansion and Pilot Phase: The evaluable population includes all patients who have measurable disease at screening, according to RECIST criteria for the relapsed solid tumor cohort and undergo a baseline disease assessment and at least one on-treatment disease assessment. Evidence of disease progression including radiological assessments, hematological assessments, death due to disease progression, and study drug withdrawal due to disease progression will be counted as post-baseline disease assessments. Evaluation for clinical response will include only those with measurable disease at baseline and evaluation of progression will be evaluated in all patients regardless of measurable disease status at baseline.

6.0 SAFETY ASSESSMENTS:

- A urinalysis for protein will be performed before starting axitinib and monthly while on it, and if more than 2+ is detected, urine protein to creatinine (UPC) ratio is to be determined (or 24 hour urine protein excretion).
- Blood pressure will be measured with every office visit, patient will be asked to keep a diary with daily record of blood pressure at home. Development of a hypertensive crisis will mandate discontinuation of the drug.
- Hematology and biochemistry will be assessed each clinic visit.
- Physical examination and vital signs will be assessed at baseline and with each clinic visit.

7.0 DOSING DELAYS/ DOSE MODIFICATIONS

Adverse Event	Action with Axitinib	Action with SLM
DLT during Run–in (see section 14.4)	NA	Permanently D/C Treatment
Grade 4 Hematologic (not controlled by standard supportive care)	Hold drug until resolved to grade 1*; restart at same dose level; if same grade 3 event re-occurs then hold until resolved to grade 1* and reduce drug as noted below**	Hold drug until resolved to grade 1*. No dose reductions.
Grade 3-4 Non-hematologic (not controlled by standard supportive care)	Hold drug until resolved to grade 1*; restart at same dose level; if same grade 3 event re-occurs then hold until resolved to grade 1* and reduce drug as noted below**	Hold drug until resolved to grade 1*. No dose reductions.
Grade 2 weight loss	Continue drug but consider dose reduction**	Continue treatment
Proteinuria > 2+ with urine protein creatinine (UPC) ratio 2.0 or greater, or 24 hour urine protein is >2 grams	Hold and repeat urine creatinine protein ratio or 24hr urine in 1 week. Resume treatment when ratio < 2.0 or under 2g/24hr; If protein >3.5g/24, hold drug and repeat 24 hr urine and creatinine clearance weekly. May resume when under 2g/24hr. Consider dose reduction if event re-occurs.	Continue treatment
Hypertension >150 systolic or >90 diastolic which is recurrent or persistent after maximum antihypertensive treatment	Interrupt until <150 systolic and <90 diastolic and reduce dose as noted below**	Continue treatment

*If patient's baseline value is > grade 1 , then interruptions should resolve to baseline value or grade 1 whichever is appropriate for the patient.

**If dose reduction from 5mg twice daily is required, the recommended dose is 3mg twice daily. If additional reduction is required, the recommended dose is 2mg twice daily.

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7.1 GENERAL GUIDELINES:

Patients who cannot resume treatment within 6 weeks due to persistent toxicities should be withdrawn from the study.

Axitinib dose can be re-escalated after symptoms improve to grade 1 and per provider discretion.

Over the course of treatment, management of some adverse drug reactions may require temporary interruption up to one week of one or both drugs per the provider discretion and after discussion with patient even if the symptoms are not grade 3/4. From previous experience in the dose escalation phase, both patients and provider found these periodic interruptions to be helpful for the patient quality of life.

Management of some adverse drug reactions may require temporary interruption or permanent discontinuation and/or dose reduction of axitinib therapy. If dose reduction from 5 mg twice daily is required, the recommended dose is 3 mg twice daily. If additional dose reduction is required, the recommended dose is 2 mg twice daily.

Patients who develop progressive disease will be off-study but will continue to be followed for survival. For equivocal findings of progression (e.g. very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

If 10% or more of the patients evaluated cumulatively every 3 months have a toxic adverse event of grade 3 or higher that was attributable to the investigational agents and that could not be alleviated or controlled within 14 days of starting supportive care, assignment of patients will be suspended until the sponsor and the data and safety monitoring committee (DSMC) have reviewed the events and determined the appropriate course of action.

8.0 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

8.1 ADVERSE EVENT REPORTING

Subject data accrued on this study will be reported in accordance with Code of Federal Regulations Title 21 (21CFR) 312.32.

This study will utilize the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 for grading all adverse events. 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 downloaded from the CTEP web site (http://ctep.cancer.gov).

8.1.1 The P.I. will notify the DSMC, IRB,, the FDA and other regulatory agencies of all serious adverse events as required by law or regulation.

8.1.2 Serious Adverse Event (AE) Reporting by investigators will be done as outlined in Table 2.

8.1.3 Definitions for reporting purposes:

Associated with the use of the drug: There is a reasonable possibility that the AE was caused by the drug. (attributions of possible, probable or definite)
Attribution: The determination of whether an AE is related to medical treatment or procedure.

Attribution Categories:

Attribution	Description
Unrelated	The AE is clearly NOT related to the intervention.
Unlikely	The AE is doubtfully related to the intervention.
Possible	The AE may be related to the intervention.
Probable	The AE is likely related to the intervention.
Definite	The AE is clearly related to the intervention.

Reporting Form and Content: For those events that meet the criteria in Table 2, please complete the Serious Adverse Event Reporting Form (MedWatch 3500a Form).

8.1.4 Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500a report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500a form
- Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B. initial, subject number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted

(The subject identifiers are important so that the new information is added to the correct initial report)

- In addition to sending the MedWatch 3500a form to the FDA, it will be submitted to the DSMC through the CRSO and the IRB if serious and related to the study drugs.

8.1.5 IRB Reporting of Serious Adverse Events and Deaths

The Protocol PI will report to the IRB all serious adverse events that are possibly, probably or definitely related to the research, as well as other reportable events, in accordance with the reporting guidelines of the IRB.

8.2 ROUTINE ADVERSE EVENT REPORTING

All Adverse Events must be reported in routine study data submissions. AEs reported through expedited SAE reports must also be reported in routine study data submissions (eCRFs).

8.3 REPORTING REQUIREMENTS FOR BASELINE ADVERSE EVENTS

Positive findings identified on baseline assessment are to be documented as a Baseline Adverse Event using CTCAE terminology and grade on the provided Baseline CRF. An expedited AE report is not required if a patient is entered on to the study with a pre-existing condition (e.g., elevated laboratory value, diarrhea). The baseline AE must be re-assessed throughout the trial and reported if it fulfills expedited AE reporting guidelines.

- If the pre-existing condition worsens in severity, the investigator must reassess the event to determine if an expedited report is required.
- If the AE resolved and then recurs, the investigator must re-assess the event to determine if an expedited report is required.
- No modification in grading is to be made to account for abnormalities existing at baseline.

8.4 ADVERSE EVENT CASE REPORT FORM

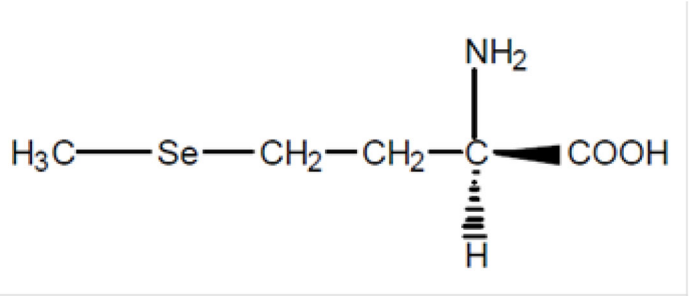
All adverse events (regardless of grade and attribution) observed while on study and for 30 days after last dose of treatment, must be recorded on the adverse event case report form in OnCore. After 30 days from last dose of treatment, only adverse events that are attributed to the study drug (possible, probable, or definite) are required to be recorded on the adverse event forms. Reporting of adverse events after 30 days from the last dose of treatment should be completed as written in Table 1.

9.0 PHARMACEUTICAL INFORMATION

9.1 SLM

Chemical Name: L-selenomethionine

Chemical Structure:



Molecular weight: 196.11 g/mol

Molecular and structural formula: C5H11NO2Se

CAS Registry Number: 3211-76-5

How supplied: Sabinsa Corporation, NJ, USA

Route of Administration: Oral

Storage: Investigational Drug will be stored in the Investigational Pharmacy at University of Iowa Hospitals and Clinics

Pharmacokinetics of L-Selenomethionine (SLM) in humans

Fakih et al conducted a phase I study to determine the dose of SLM in combination with Irinotecan required to achieve a plasma Se concentration greater than 15μM after 1 week of loading (5). SLM was administered orally, twice a day for the first 7 days followed by a daily maintenance dose. Doses were escalated as per Table 2 and trough Se blood levels were sampled on days 1, 2, 8 and 28.

Phase I escalation schema			
Dose level	SLM loading (D-7–D-1)[a] (mcg PO BID)	SLM maintenance (D1 and on) (mcg PO QD)	Irinotecan (mg/m^2) Q week (start on D1)
1	3,200	2,800	125
2	3,200	3,200	125
3	4,000	3,200	125
4	4,000	4,000	125
5	4,800	4,800	125
6	5,600	5,600	125
7	7,200	7,200	125

Table 2. Dosing schema from SLM study by Fakih et al

Table 3 shows Se plasma levels on days 8 and 28 in patients from all dose cohorts. While there was significant inter-patient variability in Se levels at day 8, all patients from dose level 5 upwards achieved the target 15µM or more. There was no significant further accumulation of Se observed at day 28 until dose level 7. Serum Se levels were measured every 6 weeks in patients who remained on study for more than 1 cycle (median number of cycles 2 (range1-8)), a plateau was reached at or around the levels achieved on day 28.

Toxicity of SLM in humans

In the Fakih study with SLM given in combination with Irinotecan, SLM was observed to be well-tolerated in all patients, with attributable toxicity limited to mild garlic-like odor in breath and urine in about 50% of the patients (5). This was seen more commonly during the induction SLM week and tended to ameliorate or disappear with prolonged treatment. No skin or nail toxicities secondary to SLM were documented. The non-tolerable dose of SLM was not defined in this study but the highest dose level administered (7200µg twice a day for 1 week followed by 7200µg daily) was deemed to be the MTD because 1 of 6 patients developed grade 3 infection, neutropenia, diarrhea, and hyponatremia.

Table 3. Plasma selenium levels on days 8 and 28 in study patients at all dose cohorts

Dose level (loading mcg BID/ maintenance mcg QD)	Pt no	Day 8 selenium level		Day 28 selenium level	
		ng/ml	μM	ng/ml	μM
DL1 (3,200/2,800)	1	785	9.94	866	10.96
DL1 (3,200/2,800)	2	1,647	20.85	1,598	20.23
DL1 (3,200/2,800)	3	1,199	15.18	No sample[a]	–
DL1 (3,200/2,800)	4	1,210	15.32	No sample[a]	–
DL1 (3,200/2,800)	5	900	11.39	1,237	15.66
DL1 (3,200/2,800)	6	1,225	15.51	1,216	15.39
DL2 (3,200/3,200)	7	633	8.01	721	9.13
DL2 (3,200/3,200)	8	1,110	14.05	No sample[a]	–
DL2 (3,200/3,200)	9	1,041	13.18	1,227	15.53
DL2 (3,200/3,200)	10	1,247	15.78	1,195	15.13
DL3 (4,000/3,200)	11	1,028	13.01	No sample[a]	–
DL3 (4,000/3,200)	12	1,298	16.43	No sample[a]	–
DL3 (4,000/3,200)	13	1,250	15.82	1,011	12.80
DL3 (4,000/3,200)	15	1,525	19.30	No sample[a]	–
DL4 (4,000/4,000)	17	1,563	19.78	No sample[a]	–
DL4 (4,000/4,000)	18	919	11.63	1,006	12.73
DL4 (4,000/4,000)	19	1,481	18.75	1,395	17.66
DL5 (4,800/4,800)	20	1,430	18.10	1,413	17.89
DL5 (4,800/4,800)	21	1,608	20.35	1,297	16.42
DL5 (4,800/4,800)	22	1,899	24.04	2,276	28.81
DL6 (5,600/5,600)	23	1,355	17.15	1,481	18.75
DL6 (5,600/5,600)	24	1,605	20.32	1,894	23.97
DL6 (5,600/5,600)	25	1,937	24.52	No sample[a]	–
DL6 (5,600/5,600)	26	1,245	15.76	1,528	19.34
DL7 (7,200/7,200)	27	NC[b]	–	1,662	21.04
DL7 (7,200/7,200)	28	2,089	26.44	2,608	33.01
DL7 (7,200/7,200)	29	2,280	28.86	No sample[a]	–
DL7 (7,200/7,200)	30	2,350	29.75	3,173	40.16
DL7 (7,200/7,200)	31	1,294	16.38	1,719	21.76
DL7 (7,200/7,200)	32	2,700	34.18	3,272	41.42
DL7 (7,200/7,200)	33	3,838	48.58	No sample[a]	–

[a] Day 29 sample was not collected

[b] Non-compliant

Pharmaceutical information

Axitinib

Physical And Pharmaceutical Properties

- Name Status: BAN, USAN, rINN
- Synonyms: AG-013736;Axitinibum
- Chemical Name: N-Methyl-2-({3-[(1E)-2-(pyridin-2-yl)ethenyl]-1H-indazol-6-yl}sulfanyl)benzamide
- Molecular Formula: C22H18N4OS
- Molecular Weight:386.5
- CAS Registry: 319460-85-0
- **How supplied: Either by the study Sponsor or as** Standard of care through subject insurance

Pharmacokinetics

Axitinib is absorbed from the gastrointestinal tract. It has a bioavailability of about 58% and is highly bound to plasma proteins. Axitinib is metabolized mainly in the liver by the cytochrome P450 isoenzymes CYP3A4 and CYP3A5, and to a lesser extent by CYP1A2, CYP2C19, and UGT1A1. Most of the dose is excreted as metabolites in the urine and feces; about 12% is excreted unchanged in the feces.

Interactions

Plasma concentrations of axitinib may be altered by the concomitant use of drugs that affect the cytochrome P450 isoenzymes CYP3A4 and CYP3A5. Strong inhibitors of these enzymes should be avoided, but if they must be used then the dose of axitinib should be reduced. Grapefruit and grapefruit juice should also be avoided. Moderate and strong inducers of these enzymes can reduce axitinib concentrations and should therefore be avoided during axitinib therapy.

Toxicities of axitinib

- The most common adverse effects of axitinib include gastrointestinal disturbances, reduced appetite, weight loss, fatigue, asthenia, dysphonia, and palmar-plantar erythrodysesthesia.
- Hypertension is also common and cases of hypertensive crisis have been reported. Blood pressure should be well-controlled before starting axitinib and monitored during therapy. Antihypertensive treatment may be needed but the dose of axitinib should be reduced if hypertension persists; axitinib should be stopped if hypertension is severe and persistent or there is evidence of hypertensive crisis. Arterial and venous thromboembolism have occurred in patients given axitinib. Reported hemorrhagic events have included cerebral hemorrhage, hematuria, and gastrointestinal bleeding. Gastrointestinal perforation and fistula formation have also been reported.
- Other adverse effects include thyroid dysfunction, proteinuria, and elevations of liver enzymes values; patients should be assessed for these before starting axitinib and monitored during therapy. There have also been cases of reversible posterior leukoencephalopathy syndrome in patients given axitinib.
- Axitinib should be stopped at least 24 hours before surgery, because of potential impairment of wound healing. (Last reviewed: 2012-03-05; last modified: 2012-03-26)

10.0 BIOMARKER AND CORRELATIVE STUDIES

10.1 BIOMARKER STUDIES:

As SLM is supposed to inhibit the expression of hypoxia inducible factors (HIF) in tumor cells, there is a possibility that downstream expression of vascular endothelial growth factors (VEGF) would be affected, thereby tumor vasculatures will be normalized and will have less leaky vessels (less permeability). Three specific markers are proposed to look at before and after treatments**: (1) HIF-α1, (2) HIF-α2, and (3) VEGF-A.**

Blood and tissue will also be collected for the determination of **miRNA** 210, 155, and Let-7b related to the disease process.

Escalation Subjects: COMPLETED on 10/23/18
SLM blood levels will be measured at baseline, on day 14, at cycles 2, 3, 4, 5, 6, and 7 then at cycles 10, 13 and 16 to correlate between blood levels and the effect on HIF-a expression. Extra blood sample of 20 ml will be collected in un-heparinized tubes, spin and stored at -80c for other biomarkers that might be added later on.

Expansion Subjects:

SLM blood levels will be measured at baseline pre dose and 2 hours post dosing, then 2 hours post dosing on day 14, at cycles 2, 3, 4 7 then at cycles 10and 13to correlate between blood levels and the effect on HIF-a expression. Extra blood sample of 20 ml will be collected in un-heparinized tubes, spin and stored at -80c for other biomarkers that might be added later on.

10.2 ARCHIVAL TUMOR TISSUE AND BIOPSY:

Archival Tumor Tissue:
A sample of archival tumor tissue, if available will be obtained for a select number of patients enrolled in the dose escalation and expansion portions of the study for biomarker studies.

Mandatory Fresh Biopsy:
Tumor biopsies should be obtained according to standard institutional procedures. The biopsies are preferred to be collected from the same lesion and the same location within the lesion at each visit of sampling. Obtain enough tissue to generate a minimum of 3 biopsy samples; one to be processed to an FFPE block for IHC, one to be processed for miRNA isolation, and one to be snap frozen for any downstream correlative testing deemed necessary. Biopsies will be obtained from Interventional Radiology and processed for research by the Tissue Procurement Core according to standard operating procedure.

For Formalin Fixation – Wrap one of the biopsy samples in Biowrap and place in a pre-labelled cassette. Place the entire cassette containing the biopsy immediately into a specimen container filled with 10% neutral buffered formalin (NBF). Transfer the specimen container containing the biopsy and cassette to surgical pathology to be processed and for embedding in paraffin according to standard institutional guidelines. Store the block at ambient temperature until testing. The biopsy should go from patient to formalin in the minimum amount of time

possible, but not to exceed 10 minutes. The formalin fixation time should be at least 6 hours, not to exceed 72 hours.

For RNAlater – Place one of the biopsy samples in a cryovial containing RNAlater solution immediately. Store the biopsy in RNAlater overnight at 4°C. The following morning, remove the supernatant and move the biopsy sample to -80°C to store until testing. The biopsy should go from patient to RNAlater solution in the minimum amount of time possible, but not to exceed 10 minutes.

For Flash Frozen – Place one of the biopsy samples in an RNase-free cryovial. Freeze in the vapor phase of LN2 immediately without submerging the tissue. Store the biopsy in LN2 vapor until testing. The biopsy should go from patient to frozen in the minimum amount of time possible, but not to exceed 10 minutes.

11.0 STUDY CALENDAR - SLM + AXITINIB FOR CLEAR CELLRCC

Evaluation	Screening Day[2] -28 to Day 0 prior to SLM Run-in	SLM Run-in Phase (+/- 2 days)		Cycle 1 (+/- 3 days)				Cycle 2 and subsequent cycles (+/- 3 days)[16,]				End of Treatment (28 days +/- 7 days) after last dose	Follow-up occurring approximately every 6 months
		Week 1	Week 2	Week 1	Week 2	Week 3	Week 4	Week 1	Week 2	Week 3[6]	Week 4		
Informed consent	X												
Verify eligibility criteria	X												
Medical History[1]	X			X									
Concurrent medication	X			X		X		X		X			
Vital signs	X			X		X		X		X		X	
Physical exam[1]	X			X		X		X		X		X	
Performance status evaluation	X			X		X		X		X		X	
ECG	X												
Lipid Panel	X												
CBC w/diff	X			X		X		X		X		X	
ALT, AST Alk phos, T.bili Na, K, calcium, magnesium, BUN, creatinine, T. protein, albumin, LDH	X			X		X		X		X[5]		X	
Erythropoietin, serum		X	Day14					X[9]					
Urinalysis	X			X				X				X	
Pregnancy test for WOCBP	X			X				X					
PT/INR, PTT	X												
Glucose	X							X[10]				X	
miRNA (blood draw)[8]		Day 1	Day14										
Fresh Tumor Tissue biopsy IHC (up to 20 patients in expansion phase and pilot phase) [13]		Day 1	Day14 (+/- 3 days)	8 weeks after first dose of SLM plus Axitinib (Cycle 3) (+/- 3 days)								X[13]	
SLM Concentration[8]		X[8]	X[8]					X[8]					
Archival tumor tissue		X[12]											
Additional blood for storage [8]		X[8]	X[8]					X[8]					

CT +/- Bone scan[3] Expansion Phase/Pilot Phase		Beginning at Cycle 5 Day 1 Imaging will be repeated every 8 weeks +/- 5 days.[14,17]											
CT +/- Bone scan[3] Escalation Phase	X[7]	Scans will be repeated every 8 weeks +/- 5 days, starting 8 weeks after first dose of SLM plus Axitinib, as clinically indicated.[17]											
Dual Energy CT[15] Expansion/Pilot Phase	X[7]		Day 14	8 weeks after first dose of SLM plus Axitinib (Cycle 3)									
SLM Dosing													
Axitinib Dosing													
Adverse Event Assessment													
Safety Phone Call			X		X								X[11]

[1] History and physical exam will be performed by physician and/or midlevel provider or designated fellow. To include cardiac risk factors, smoking history, family history of cardiac disease, use of aspirin.
[2] Events may be performed Day 1 of SLM run-in if resulted/reviewed prior to dosing.
[3] CT scan (with or without Contrast) of chest, abdomen and pelvis with or without bone scan based on provider's judgment. Bone Scans will only be repeated if lesion is present at baseline.
[5]These labs will be drawn as clinically indicated.
[6]Visit 3 for Cycle 2 and beyond will only occur if clinically indicated.
[7] Screening CT to be performed within 28 days from SLM Day 1.
[8] See Table 20.0 for Escalation patients. See Table 20.1 for Expansion patients. See Table 20.2 for Pilot Phase patients.
[9] Cycle 3 only
[10] Blood drawn for glucose testing every even cycle beginning with Cycle 2.
[11]Patients will be followed up, until they are lost to follow-up or death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed for resolution or stabilization of the adverse event as well. Follow up off study will be performed using telephone contact, correspondence with treating physicians, and death records as necessary to update vital status at least every 6 months.
[12]Archival tumor tissue will be obtained, if available and if subject agrees.
[13] Fresh research tissue biopsies will occur in up to 20 subjects enrolled into the Expansion Phase and pilot phase, that the PI deems as safe and tumor is accessible. An additional fresh research tissue biopsy may occur at progression for subjects that the PI deems as safe and tumor is accessible.
[14] CT +/- Bone scans will be performed as clinically indicated beginning at Cycle 5, Day 1. Prior to Cycle 5, Day 1Dual Energy CT's will be performed.
[15] For subjects in expansion phase and pilot only Dual Energy CT's will follow protocol as outlined in Section 22.
[16] Per PI or Sub-I discretion, subjects may be seen at odd cycles after Cycle 11 is completed (Cycle 11, 13, 15, 17, etc). Subjects who are allowed to be seen at odd cycles will be dispensed with 8 weeks of study drug and asked to contact the research team with any issues or concerns between visits. (if patient is seen at odd cycles then no assessments are required at even cycles. Labs may be obtained locally.)
[17] If subject is asymptomatic, bone scans may be done every 4 months.

12.0 MEASUREMENT OF EFFECT

12.1 CRITERIA FOR EVALUATION OF THERAPY EFFECTIVENESS

Tumor response and regrowth can frequently be difficult to measure directly. Three CT scans performed will be dual energy at the following time points: Baseline, Day 14 and Cycle 3. Serial CT scans will be performed at all additional time points based on tumor types may provide a guide to the actual course. Time interval to progression will be measured from registration until deterioration is documented by the individual investigator using these guides.

- **Progression-free survival** will be measured from the start of cancer treatment until the first occurrence of progression or death.
- **Overall survival** will be measured from the start of cancer treatment until death.
  The quality of survival will be measured by functional classification and performance status

Patients will be followed both clinically and radiographically for evidence of tumor progression. Post-treatment scans will be compared to the baseline CT scan and responses will be assessed using response evaluation criteria in solid tumors (RECIST 1.1).

12.2 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response every 8 weeks (every other cycle) by CT scans for body images.

12.2.1

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the criteria.

- Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with SLM. Toxicities will be measured using the CTCAE criteria, version 4.03
- Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle 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 1 will also be considered evaluable.)
- Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

12.2.2 Disease Parameters

- Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray, as ≥10 mm with CT scan, or ≥10 mm with calipers by clinical exam. 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 ≥15 mm 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.

- Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

- Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

  Cystic lesions thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

- Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 3 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

- Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 3 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

12.2.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of 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 is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

- Conventional CT and/or MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.

- Dual Energy CT:
  - Contrast: 50% saline mixed with 50% 370mg/ml Iopamidol delivered at 4ml/s (4ml/s (35 s+ 5s) = 160ml of which 80ml is contrast) with delay of 35s (for chest) and 70s (for abdomen pelvis)
  - Positioning: Cranialcaudal acquisition with scan coverage (DFOV) from pubic symphysis to apex of lungs (DFOV to match prior visit). Acquisition at coached total lung capacity (TLC) (recorded for DECT)
  - Acquisition: CHEST: The spiral DECT contrast scan with dose modulation with tube A: 120 ref mAs/80kV, tube B: 67 ref mAs/Sn150 kV, with a pitch of 0.55 and rotation time of 0.25sec. The CTDIvol is assumed to be 4.28mGy, with total DLP of 143.2mGy*cm for a 30cm scan.
  - Acquisition: ABDOMEN: The spiral dual energy contrast abdomen/pelvis scan protocol uses dose modulation with tube A: 190 ref mAs/100kV, tube B: 95 ref mAs/Sn150 kV, with a pitch of 0.55 and rotation time of 0.25sec. The CTDIvol is assumed to be 10.41mGy, with total DLP of 553.7mGy*cm for a 50cm scan.
  - Reconstruction: R1: 0.75 x0.5; R2: 2.0 x 1.4, Qr40(Admire 5), FOV to match prior visits, Energy Series: A, B and M. Keep Raw data.

- Bone Scan: Will be ordered if the patient has new bone pain, or with elevated alkaline phosphatase, and in patients with known bony metastasis, looking for any new or worsening old bony lesions.

- PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

12.3 RESPONSE CRITERIA.

12.3.1.1 Evaluation of Target Lesions

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

- Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

- Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

12.3.1.2 Evaluation of Non-Target Lesions

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumormarker level. All lymph nodes must be non-pathological in size (<10 mm short axis).
  Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

- Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

- Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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

12.3.1.3 Evaluation of Best Overall Response

- The best overall response is the best response recorded from the start of the treatment untildisease 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 (Table 4).

12.3.1.4 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).
  The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

- 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, including the baseline measurements.

12.3.1.5 Progression-Free Survival

- PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

Table 4: **For Patients with Measurable Disease (i.e., Target Disease)**

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** 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.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*

Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

12.3.1.6 Response Review

Patients with measurable disease will be assessed by standard criteria. The purpose of tumor measurements will be to assess benefit to the patients from treatment and to determine appropriateness for continuing on study. For the purposes of this study, patients should be re-evaluated every week to every six weeks according to the study guidelines and undergo imaging studies as highlighted in the study calendar. Following documentation of an objective response, confirmatory scans will also be obtained a minimum of eight weeks later.

13.0 DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 8 (Adverse Events: List and Reporting Requirements).

13.1 REGULATORY COMPLIANCE/GOOD CLINICAL PRACTICES

This study will be conducted in accordance with the following regulations and guidelines, to include but not limited to:

- Declaration of Helsinki (October 2000)
- Current ICH Guideline for Good Clinical Practice
- 21 CFR 50: Protection of Human Subjects
- 21 CFR 54: Financial Disclosure by Clinical Investigators
- 21 CFR 56: Institutional Review Boards
- 21 CFR 312: Investigational New Drug Application

13.2 REGULATORY DOCUMENTATION

Prior to study start-up, investigators will submit the following documents to the sponsor, as outlined in the Essential Documents Section 8.0 of the ICH Guidelines for Good Clinical Practice to include but not limited to:

- Signed Confidentiality Agreement
- Signed Clinical Trial Agreement, if applicable
- Up-to-date signed and dated Curriculum vitae and copies of medical licenses for Principal and one sub/co-investigator with CVs for all investigators to be submitted promptly
- Financial Disclosure form for Principal and one sub/co-investigator with financial disclosure forms for all investigators to be submitted promptly
- FDA Form 1572
- IRB approval to conduct the study: IRB-approved informed consent form,
- Name and address of the IRB with the statement that it is organized and operates according to GCP and the applicable laws and regulations
- IRB membership roster
- Local laboratory certifications, its name and address
- Local laboratory normal ranges (a dated copy for tests to be performed during the study).
- Financial agreement, if applicable.
- Signed and dated Investigator Agreement page of the final protocol and amendments, where applicable.

13.3 INSTITUTIONAL REVIEW BOARD (IRB)

This Trial will be undertaken only after full approval of the protocol and addenda has been obtained from a local IRB and a copy of this approval has been received by the sponsor. The IRB must be informed of all subsequent protocol amendments issued by the sponsor. Reports on and reviews of, the trial and its progress will be submitted to the IRB by the investigator at intervals set force in its guidelines.

13.4 INFORMED CONSENT

Each subject must give written consent and sign other locally required documents after the nature of the study has been fully explained. The informed consent form must be signed prior to performanceof any study-related activity with the exception of baseline imaging (if already obtained within 14 days prior to starting study treatment) and baseline lab studies (if already obtained within 7 days prior to starting study treatment). The informed consent form that is used must be approved both by the sponsor and by the reviewing IRB. The Informed Consent should be in accordance of the Declaration of Helsinki, current International Conference on Harmonization (ICH) and Good Clinical Practices (GCP) guidelines.

13.5 ADMINISTRATIVE REQUIREMENTS

13.5.1 Protocol modifications

The investigator will not modify this protocol without obtaining permission from the sponsor. All protocol amendments must be issued by the sponsor, signed and dated by the investigator, and should not be implemented without prior IRB approval, except where necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the trial (e.g., change in monitor(s), change of telephone number(s).

In situations requiring a modification, the investigator or other physician in attendance will contact the medical monitor by fax or telephone. This contact must be made prior to implementing any departure from protocol. Contact with the sponsor must be made as soon as possible in order to outline an appropriate course of action.

13.5.2 Record Retention

In compliance with the ICH/GCP guidelines the investigator/institution will maintain all CRFs and all source documents that support the data collected from each patient, and all trial documents as specified in Essential Documents for the Conduct of a Clinical Trial and as specified by the applicable regulatory requirement(s).

The investigator/institution will take measures to prevent accidental or premature destruction of these documents. Essential documents must be retained until at least two years after the last approval of a marketing application in an ICH region or at least two years have elapsed since the formal discontinuation of clinical development of the investigational product.

These documents will be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

13.5.3 Data Management

Source documents will be developed, as needed, to capture necessary clinical data. The source documents are to be completed as soon as possible after the subject's visit, so that they reflect the latest observations for subjects participating in the trial.

All required clinical data will then be entered into the Clinical Trials Management System, OnCore. Electronic case report forms (eCRF) will be designed and developed to capture study specific data. All corrections or revisions to the eCRFs must be made by authorized personnel and an audit trail will be maintained.

All finalized data will be reviewed by the Investigator. Data recorded in the eCRFs will be retained by the site in accordance with FDA regulations.

13.5.4 Monitoring

Monitoring will be completed based on the Data and Safety Monitoring Plan in Appendix B.

13.5.5 Data Quality Assurance

Steps to be taken to assure the accuracy and reliability of data include the selection of qualified investigators, review of protocol procedures with the investigator and associated personnel prior to the study, and periodic monitoring visits by the sponsor. CRFs will be reviewed for accuracy and completeness by the CRO during on-site monitoring.

13.5.6 Use of Information and Publication

All information on SLM, patent application, manufacturing process and basic scientific data supplied by the sponsor to the investigator and not previously published is considered confidential and remains the sole property of the PI.

The investigator agrees to use this information only to accomplish this study and will not use it for other purposes without the sponsor’s written consent.

The investigator understands that the information developed in the clinical study will be used by Sponsor in connection with the continued development of SLM and thus may be disclosed as required to other clinical investigators or government regulatory agencies.

To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the sponsor with all data obtained in the study.

14.0 STATISTICAL CONSIDERATIONS

14.1 INTRODUCTION

This is a Phase I safety clinical trial with expansion cohort for assessing preliminary efficacy of the combination of axitinib and selenomethionine (SLM) for adult patients with advanced clear cell carcinoma (CCRCC). The Escalation Part 1 will determine the MTD of the combined treatment to be used for the proof of principle. The expansion cohort will be used to assess preliminary efficacy.

Summary statistics for continuous variables will include: mean, standard deviation, median and range. Categorical variables will be presented as frequency counts and percentages. Progression-free and overall survival will be summarized by Kaplan-Meier medians and survival plots. Data listings will be created to support tables and present data. The preliminary efficacy analysis will be conducted on the efficacy evaluable population and safety analysis will be performed on the safety population. SAS 9.3 or higher will be used for data analysis.

14.2 STUDY OBJECTIVES

The primary objectives of this study are:

- To characterize the safety profile of combining SLM and axitinib in advanced metastatic CCRCC and;
- To define the dose of SLM in combination with a standard dose of axitinib for phase 2 trial.

The secondary Objectives:

- To determine that the dose of SLM administered is sufficient to alter the expression levels of HIF1a, HIF2a, VEGF and their associated micro-RNAs 210, 155, and LET7b.
- To obtain preliminary estimates of efficacy in terms of:
  - Progression free survival (PFS),
  - Objective response rate (SD+PR+CR), and
  - Overall survival in patients with advanced metastatic CCRCC.

The exploratory objective is to define the PD effects of SLM.

14.3 ANALYSIS OF STUDY ENDPOINTS

14.3.1 Analysis of primary endpoint

a. The safety profile of the SLM and axitinib combination will be assessed by listing the overall incidence of AEs. The AEs will be summarized and classified by body system and by treatment group. The type, incidence, severity, and causality of each AE, the duration of the event, and any required treatment interventions will be tabulated. Physical examination results will be presented in the patient data listings. The DLT will be listed per dose level and treatment along with overall frequencies. The data from the expansion part will be used for this part of safety and tolerability assessment.

b. (Recommended Part 2 dose)

Dose level 1 (4000 µg) will be the starting dose in the standard 3+3 dose escalation design. Three patients will be recruited for Dose level 1.

The next three patients will be recruited for Dose level -1 (2500 µg). While none of the three patients in Dose level 1 experienced a DLT, there were significant side effects.
If 0 of three patients at Dose level -1 experiences a DLT related to SLM, then dose escalation will proceed to the next dose level (3000 µg).

If one of the three patients at Dose level -1 experiences a DLT related to SLM, then three more patients will be treated at the same dose. If exactly one out of six patients experiences a DLT, then dose escalation will proceed to the next dose (3000 µg) after discussion at the weekly SLM meeting.

If two of the first three patients experience DLT, then the dose will de-escalate to the lower dose (1500 µg).

The next patient can be enrolled after the previous patient's Day 28 assessment, provided there are no DLTs. The minimum number of patients required is 6 and the maximum is 12.

14.3.2 Analysis of secondary endpoint

a) The expression levels of HIF1a, HIF2a, VEGF and their associated micro-RNAs 210, 155, and LET7b will be evaluated to check if any is altered at each of the dose levels administered.
b) The following will be provided for the treatment combination:
   - Point estimate of median PFS time and 95% confidence interval by Kaplan-Meier along with survival plots.
   - Point estimate along with 95% confidence interval of the objective response rate (definedas SD+PR+CR, estimated).
   - Point estimate along with 95% confidence interval of the overall survival (OS).

14.4 SAMPLE SIZE AND POWER:

The number of patients to be enrolled in the Escalation Part 1 will depend upon the observed safety profile. The minimum number of patients required is 6 and the maximum is 12.

Of greatest interest for the expansion part 2 is to assess the progression free survival (PFS) for patients treated the dose of SLM in combination with a standard dose of axitinib in advance metastatic CCRCC. Historic median survival for patients within similar subgroup of advanced metastatic CCRCC is reported in the literature to be around 8 months. The goal of the pilot is to have a firsthand rough estimate of the median survival in our study population and define hypotheses for a subsequent larger trial. Consequently, only a small sample would be required. To justify our sample, we will be operating under the assumption that the median survival will increase to 14 months in our treated population and that 90% upper confidence intervals will be constructed for the PFS (which would be equivalent to testing at 10% significance level, if statistical tests were to be carried). This may not be a stone-cut hypothesis; but reasonable for pilot justification. For the statistical analysis, exponential survival will be assumed and the associated survival rate estimated with maximum likelihood methods. A Wald statistic will be used to test this imperfect null hypothesis that median survival is equal to 8 months versus the one-sided alternative that it is greater than 14 months. With the planned enrollment of 19 patients accrued over 18 months and a total follow up for 14 months. The study will have 80% power to detect an increased survival of 6 months. The reason for this power justification is to put things into perspective and give an idea that one can actually glean some information out of this study—information that will serve as hypothesis generating for a larger trial. Estimates will be reported along with confidence intervals.

Pilot phase: Extra cohort with BSA based dose:

The primary objective of this pilot dose-finding study is to characterize the dose-concentration relationship and estimate the effective administered dose of selenium necessary to achieve the target blood concentration range informed by preclinical data. Preliminary data from the initial trial indicate a single fixed dose of selenium conferred significant variability in patient blood selenium concentrations at the end of the 14-day run-in. Patient body surface area (BSA) was found to be a contributing factor with patients of lower BSAs more frequently having higher blood selenium concentration, although none of which were within the targeted range of 35-45 µM. In light of these findings, a modified dosing scheme is being investigated which varies the dosage of selenium by BSA within a dose level to ensure less variability in the dose per m^2 administered.

Continual Reassessment Method

Dose escalation for this pilot study will be conducted using a continual reassessment method (CRM) in which the probability of exceeding a blood selenium concentration of 45 µM on Day 14 is being modeled. Prior probabilities of exceeding a blood selenium concentration of 45 µM on Day 14 were estimated based on preclinical and preliminary data from the initial trial. A one parameter logistic model with intercept set at 3 and an initial value of 1 for the slope will be used to estimate the dose-concentration relationship through sequential recursive Bayesian assessment. The target probability of exceeding 45 µM is ≤20%.

	Dose Level[a]				
BSA	1	2	3[b]	4	5
1.50-1.79	2000	3000	4000	5000	6000
1.80-2.19	3000	4000	5000	6000	7000
2.20-2.49	4000	5000	6000	7000	8000
π_{45}[c]	0.001	0.01	0.10	0.20	0.30

[a]All doses are mcg BID Day 1-14 then daily until progression.
[b]Starting dose level.
[c]Probability of exceeding 45 µM (prior)

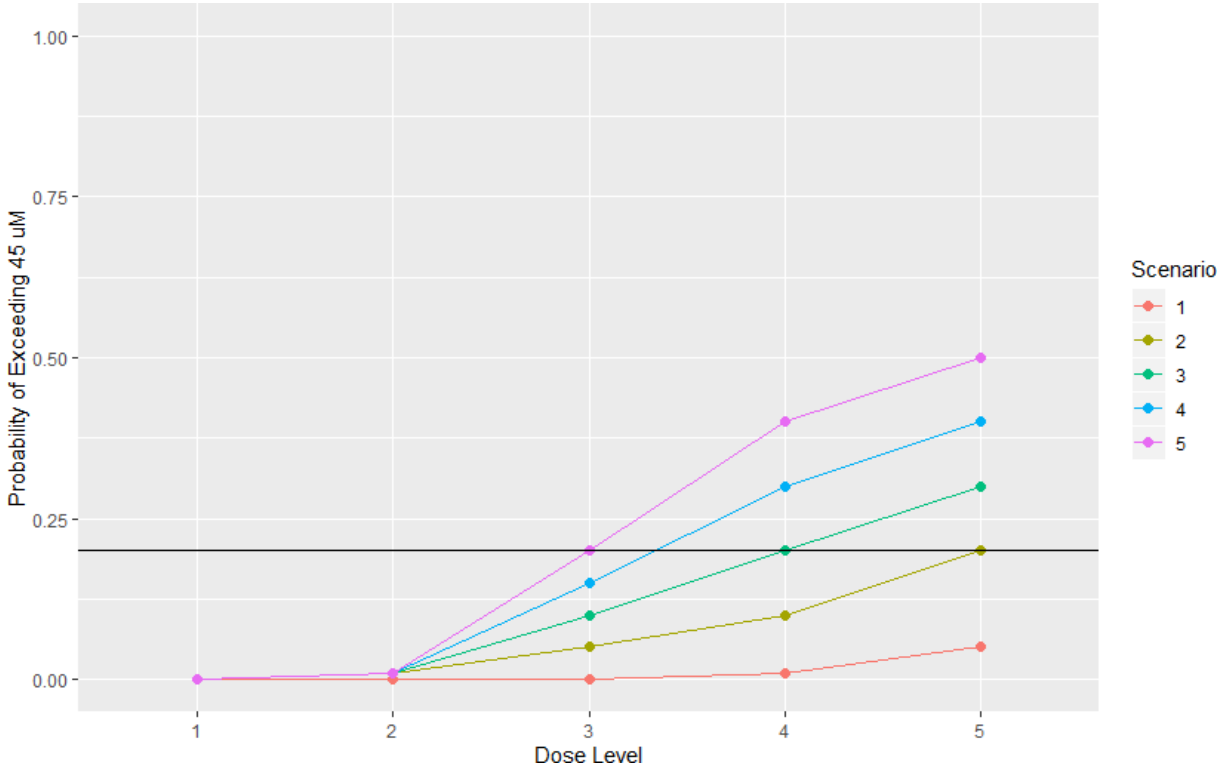
Dosing will begin at dose level 3. Each cohort will enroll 2 evaluable patients. After each patient has completed the Day 14 evaluation, the following information will be provided to the statistical research team:

1. Blood selenium concentration on Day 14
2. DLTs, defined as any of the following treatment-related events occurring between Day 1 and 14 irrespective of outcome:
    a. Clinically significant non-hematologic toxicity of Grade 3 or greater not related to underlyingmalignancy.
    b. Severe hematological toxicity (Grade 4 neutropenia, Grade 3-4 febrile neutropenia, or Grade 4 thrombocytopenia).
    c. Grade 4 nausea, vomiting, diarrhea, or electrolyte imbalances or Grade 3 nausea, vomiting, diarrhea and electrolyte imbalances lasting greater than 48 hours despite optimal medicalintervention.

At which time, the dose-concentration model will be updated to obtain the recommended dose level for the subsequent cohort. Dose escalation will proceed one dose level at a time; skipping dose levels is not permitted.

Simulations

Simulations were conducted to evaluate the performance of the proposed design across various scenarios. More specifically, several potential underlying truths of the dose-concentration profiles were considered, and how the trial behaved in each of those scenarios was evaluated. Scenarios under consideration are those represented in the figure and table below. For each of the scenarios, simulations are based on the following specifications: cohorts of size 2 will enter the trial, a total of 10 patients will be used for each simulation, 1000 Monte Carlo replications are used, a one-parameter logistic model with starting parameter of 3 for the intercept and an initial slope of 1, no skipping dose levels, and starting at dose level 3.



	Dose Level				
	1	**2**	**3**	**4**	**5**
True Scenario 1	0.001	0.001	0.001	0.010	0.050
True Scenario 2	0.001	0.010	0.050	0.100	0.200
True Scenario 3 (Prior)	0.001	0.010	0.100	0.200	0.300
True Scenario 4	0.001	0.010	0.150	0.300	0.400
True Scenario 5	0.001	0.010	0.200	0.400	0.500

π_{45} ≤20% are in green and >20% in red.

The operating characteristic using simulations for each scenario are summarized below.

		Dose Level				
		1	2	3	4	5
True Scenario 1:	% Selection	0.00	0.20	0.30	2.20	97.30
	% Subjects Treated	0.04	20.24	20.26	20.76	38.70
	# Subjects Treated	0.00	2.02	2.03	2.08	3.87
	Average Toxicities	0.00	0.00	0.01	0.02	0.21
	True Probabilities	0.001	0.001	0.001	0.010	0.050
True Scenario 2:	% Selection	0.00	2.70	6.50	22.50	68.30
	% Subjects Treated	0.52	23.80	24.92	22.98	27.78
	# Subjects Treated	0.05	2.38	2.49	2.30	2.78
	Average Toxicities	0.00	0.02	0.12	0.23	0.56
	True Probabilities	0.001	0.010	0.050	0.100	0.200
True Scenario 3:	% Selection	0.00	4.30	20.00	31.50	44.20
	% Subjects Treated	0.62	25.74	29.82	24.32	19.50
	# Subjects Treated	0.06	2.57	2.98	2.43	1.95
	Average Toxicities	0.00	0.02	0.30	0.48	0.59
	True Probabilities	0.001	0.010	0.100	0.200	0.300
True Scenario 4:	% Selection	0.00	7.20	33.70	36.30	22.80
	% Subjects Treated	0.74	28.50	34.00	22.76	14.00
	# Subjects Treated	0.07	2.85	3.40	2.28	1.40
	Average Toxicities	0.00	0.02	0.49	0.65	0.60
	True Probabilities	0.001	0.010	0.150	0.300	0.400
True Scenario 5:	% Selection	0.10	13.00	48.20	27.60	11.10
	% Subjects Treated	1.34	32.94	36.02	21.36	8.34
	# Subjects Treated	0.13	3.29	3.60	2.14	0.83
	Average Toxicities	0.00	0.03	0.70	0.86	0.41
	True Probabilities	0.001	0.010	0.200	0.400	0.500

π_{45} ≤20% are in green and >20% in red.

Additional Safety Considerations

Preliminary data from the initial trial demonstrated the doses explored thus far were safe. In the Phase I dose-finding study of selenium in combination with irinotecan, all dose levels (max 7,200 mcg) evaluated were found to be safe but the upfront selenium run-in period was a week shorter than the current proposed dosing schedule (BID for 7 days vs BID for 14 days). As an additional safety precaution, patient accrual will pause if any DLTs are evidenced between Day 1 and 14 until the data and safety monitoring committee (DSMC) have reviewed the events and the appropriate course of action has been determined.

14.5 TREATMENT RANDOMIZATION AND BLINDING

There is no randomization for this study.

14.6 ANALYSIS POPULATIONS

The safety population will consist of all subjects receiving at least 1 dose of study medication. All subjects who received at least one dose of study treatment and had at least 1 post-baseline response evaluation will be included in the efficacy analysis set. Following completion of the study, best response will be determined for each subject in accordance with RECIST v.1.1 guidelines and the objective response rate presented for each dose cohort.

14.6.1 Patient Disposition

The number and percent of patients entering and completing the clinical study will be presented by dose and cohort and study Part.

14.6.2 Demographic Information and Baseline Characteristics

Demographic and baseline characteristic will be descriptively summarized by study phase and dose treatment.

14.7 PROTOCOL DEVIATION

Protocol deviation will be listed by patient

14.8 INTERIM ANALYSIS

No formal interim analysis is planned for the study. However, reports will be generated post the dose escalation part 1 of the study and adverse events and toxicity reports will be monitored.

14.9 HANDLING OF MISSING DATA

The data for the efficacy endpoint will be analyzed as Intention to treat (ITT) and according to protocol (ATP). Primary endpoint estimate and associated hypothesis testing will be provided for both ITT and ATP analyses. The OS rate as a secondary endpoint analysis will be provided for ITT and ATP.

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16.0 APPENDIX A

APPENDIX A

Performance Status Criteria

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

17.0 APPENDIX B: HCCC CLINICAL TRIAL DATA AND SAFETY MONITORING PLAN (DSMP)*

Date: 03/12/2019

IRB#: 201507716

IND#: Exempt #126767

Title: A Therapeutic Trial for Safety and Preliminary Efficacy of the Combination of Axitinib and Selenomethionine (SLM) for Adult Patients with Advanced Metastatic Clear Cell Renal Cell Carcinoma (CCRCC)

PI: Yousef Zakharia, MD

***** *All investigator-initiated protocols will be subject to ongoing monitoring of accrual, subject eligibility, protocol modifications and continuing reviews. Active studies will be audited for the DSMC by the CRSO, following guidelines based on level of risk to subjects. Audits will be conducted by reviewing subject files provided by clinical research coordinators, as well as original source documentation provided by online medical records and research pharmacists. The CRSO will review adverse events, eligibility of subjects, and adherence to the IRB- and PRMC-approved protocol. Protocols found to have discrepancies will be require a response from the PI and an action plan for correcting identified deficiencies. The DSMC will provide a schedule of audit and report dates if requested.*

Type of Clinical Trial:

- ❒ Investigator-initiated (UI/HCCC)
- ❑ Investigator-initiated, participating site
- ❑ Pilot study
- ❒ Phase I
- ❑ Phase I/II
- ❑ Phase II
- ❑ Phase III
- ❑ Compassionate-use/Expanded Access
- ❒ Interventional Treatment
- ❑ Interventional Non-Treatment
- ❑ Non-Interventional

Study risk-level:

- ❑ Level 1—low risk of morbidity or death, * <1% of death or any adverse event
- ❑ Level 2—risk of death* <1% or any adverse event 1% – 5%
- ❑ Level 3—risk of death* 1% – 5% or grade 4 – 5 SAE 1% – 5%
- ❒ Level 4—risk of death* >5% or grade 4 – 5 SAE >5%
- ❑ Drugs being used on a "compassionate" basis

***** *Risk of death" refers specifically to 100-day treatment-related mortality*

Reporting and Monitoring Requirements:

All institutional investigator initiated trials (IITs), regardless of assigned risk level are subject to routine DSMC monitoring activities which may include but are not limited to review of signed consent documents, eligibility and adverse event reporting.

All institutional IITs have the following **reporting requirements** as part of their DSMP:

- Register all subjects in HCCC's Clinical Trial Management System, OnCore
- Document Adverse Events
- Document protocol deviations
- Provide an annual progress report to the DSMC via OnCore data export

Selected monitoring strategy based on risk-level:

Risk Level 4

Interventional treatment trials involving investigational agents or devices with a risk of death* (>5% or grade 4 – 5 SAE >5%), e.g. all investigator initiated INDs, most Phase I/II trials, gene therapy, gene manipulation or viral vector systems high-risk clinical procedures if performed solely for research purposes. The use of a new chemical or drug for which there is limited or no available safety data in humans.

Study Safety Review

An independent study monitor and/or the DSMC Chair (or designee), will review study data (provided by the PI/available in OnCore) and communicate with the PI at least biannually. A copy of this communication will be forwarded to the DSMC and PRMC Chairs.

Additional Reporting Requirements:

- A scanned copy of the completed eligibility checklist, with screening information and PI signature, will be attached in OnCore for ongoing review by DSMC staff.
- Serious adverse events will be entered directly into an OnCore SAE report by the research team. OnCore will send an automatic notification to the DSMC Chair/acting Chair and staff for review.
- The DSMC utilizes a risk-based monitoring approach. The trial's research records will be monitored at minimum twice per year. Monitoring may be done more frequently depending on the protocol, risks to subjects, reported serious/adverse events, patient population and accrual rate. Records for a minimum of 25% of subjects will be monitored for the entire study.

  Monitoring will involve the following:

  - review eligibility of patients accrued to the study,
  - check for the presence of a signed informed consent,
  - determine compliance with protocol's study plan,
  - determine whether SAEs are being appropriately reported to internal and external regulatory agencies,
  - compare accuracy of data in the research record with the primary source documents,
  - review investigational drug processing and documentation,
  - assess cumulative AE/SAE reports for trends and compare to study stopping rules.

Routine Adverse Event Reporting

For non-serious Adverse Events, documentation must begin from the first day of study treatment and continues through the 30 day follow-up period after the last dose of the investigational agent**.**

Collected information should be recorded in the electronic/Case Report Forms (eCRF/CRF) for that subject. A description of the event, its severity or toxicity grade (according to NCI's Common Toxicity Criteria (CTCAE)), onset and resolved dates (if applicable), and the relationship to the study drug should be included. Documentation should occur in real time. The principal investigator has final responsibility for determining the attribution of the event as it is related to the study drug.

Serious Adverse Event Reporting

For any experience or condition that meets the definition of a serious adverse event (SAE), recording of the event must begin after signing of the informed consent and continue through the 30 day follow-up period after treatment is discontinued.

Investigators must report to the DSMC any serious adverse events (SAE), whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64). SAEs must be reported via an OnCore SAE Report within 24 hours of learning of the event.

An adverse event is considered **serious** if it results in ANY of the following outcomes:

1. Death
2. A life-threatening adverse event
3. An adverse event that results in inpatient hospitalization OR prolongation of existing hospitalization for ≥24 hours
4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. A congenital anomaly/birth defect.
6. Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

FDA Reporting Requirements (for Sponsor-Investigators)

It is the responsibility of the IND sponsor-investigator to comply with IND safety reporting as set forth in the Code of Federal Regulations, Section 312.32. This responsibility includes providing an annual IND report to the FDA.

All IND safety reports must be submitted on Form 3500A and be accompanied by Form 1571. The type of report (initial or follow-up) should be checked in the respective boxes on Forms 3500A and 1571. See Instructions for

completing Form 3500A. Please note all instance of UIHC, location, and faculty / staff should be redacted from supporting documentation and the 3500A.

The submission must be identified as:

- "IND safety report" for 15-day reports, or
- "7-day IND safety report" for unexpected fatal or life-threatening suspected adverse reaction reports, or
- "Follow-up IND safety report" for follow-up information.

For detailed explanation of the above definitions, requirements, and procedures related to IND application safety reports and the responsibilities of IND applications sponsors with regard to such reporting, refer to Guidance for Industry and Investigators: Safety Reporting Requirements for INDs and BA/BE Studies (PDF - 227KB)

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known (grading the event per CTCAE)
- Supportive laboratory results and diagnostics
- Sponsor-Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Data Monitoring and Management

Subject Registration

All studies that undergo PRMC review and/or utilize HCCC Clinical Research Services (CRS) resources are required to register subjects in OnCore. Each subject registration includes the following:

- The subject's IRB approved (version date) consent form and the date of their consent
- Date of eligibility and eligibility status (eligible, not eligible)
- On study date and subject's disease site (and histology if applicable)
- On treatment date (if applicable)

All subject registration information is expected to be entered into OnCore within **2 (two) business days** after the subject's study visit.

Subject Data

For HCCC investigator initiated trials, research staff are responsible for entering subject study data (data collection) into OnCore electronic case report forms (eCRFs). These eCRFs must be approved by the PI and statistician prior to study activation to ensure sufficient and necessary data acquisition. All information entered into eCRFs will be traceable to the source documents which are generally maintained in the subject's file.

eCRF data entry needs to be timely and should be entered into OnCore as soon as possible but no later than **14 (fourteen) business days** after the subject's visit, including adverse events, tumor measurements, administration of study medication, concomitant medications, labs, and vitals. Physical exam assessments must be entered no later than **14 (fourteen) business days** following completion of the physician's clinic note in the medical record.

Timely data entry facilitates remote monitoring of data, allows the data to progress appropriately through the data cleaning process, and helps prevent a backlog of data queries.

Forms Monitoring

OnCore eCRF data are monitored on a routine basis (dependent on accrual) to ensure all data are entered completely, accurately, and within time requirements outlined above. The assigned DSMC monitor will coordinate and complete the data monitoring review. When the time comes to monitor a study (based on patient accrual and assigned risk level of trial) the monitor arranges for a selection of cases to be reviewed from among the subjects registered in OnCore. As part of the forms monitoring process, the assigned monitor will issue queries via OnCore (linked to the eCRF) to resolve missing, incomplete, and/or incorrect information. A member of the research team is expected to respond to these monitoring queries within **14 (fourteen) business days.**

The monitoring process can often identify misunderstandings or deficiencies in the written, research protocol requirements earlier in the study process and thereby improve data quality and reduce rework.

Final Reports

A summary of each subject's data record is continually available to the PI, research staff, and DSMC from OnCore's Biostat Console. The availability of this information is a valuable tool for the preparation of final reports and manuscripts as well as ongoing deficiency reports.

18.0 APPENDIX C: SLM RUN-IN PHASE

Subject Initials: _____

Cycle Start Date:_____/____/_____

INSTRUCTIONS TO THE PATIENT:
You will take one Selenium (SLM) capsule_____mcg twice a day for 14 days.
Record the date and time (HH:MM) you took study your medication in the space provided below.
If you have any comments or notice any side effects, please record them in the Comment column.
Please bring your medication bottles and this diary with you at each visit to review with the study team.

If you have any questions or concerns, please call your study nurse **Janelle Born 319-356-4797** (usual business hours M-F).
After hours and weekends, please call the hospital operator at 319-356-1616 and ask for the cancer doctor on-call.

Date	Day	SLM AM Dose time	SLM PM Dose time	Axitinib AM Dose time	Axitinib PM Dose time	Comments
	1			[redacted]		
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
	13					
	14					

PATIENT DRUG DIARY, Cycle 1 and beyond

Subject Initials: _____

Cycle Start Date:____/____/_____ **Cycle: _____**

INSTRUCTIONS TO THE PATIENT:
Dose: Selenium (SLM)_____mcg **once** daily, and Axitinib (Inlyta)_____mg **twice** daily.
Record the date and time (HH:MM) you took study your medication in the space provided below.
If you have any comments or notice any side effects, please record them in the Commentcolumn.
Please bring your medication bottles and this diary with you at each visit to review with the study team.

If you have any questions or concerns, please call your study nurse **Janelle Born 319-356-4797** (usual business hours M-F).
After hours and weekends, please call the hospital operator at 319-356-1616 and ask for the cancer doctor on-call.

Date	Day	SLM AM Dose time	Axitinib AM Dose time	Axitinib PM Dose time	Comments
	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
	11				
	12				
	13				
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	27				
	28				

19.0 SLM PROCEDURE LAB MANUAL

+ Test	Tube	Size	Number	Instructions	Storage / Shipment	Label As
Selenium (Refer to Protocol)	EDTA (Purple)	**3 ml**	1	Refrigerate	Ship ambient to Ankeny[1]	Pre / Post SLM level
miRNA (Collected only on Day 1 and Day 14) **6 ml tube to be used**	EDTA (Purple) ——— (**4 ml** remaining; save for future studies)	**2 ml** Whole blood --- **2 ml** whole blood **2ml** plasma	1 1 1	Transfer unspun whole blood to a **(1 cryovial)** Freeze Transfer unspun whole blood to**(1 cryovials,)** Freeze Spin tube at 3000 × RPM/3.0 G for 10 mins, Collect plasma into**(1 cryovials)**	Ship Frozen[2] , Store at -80 °C, ship in batches Ship Frozen[3], Store at -80 °C Ship Frozen[3], Store at -80 °C	miRNA Whole Blood Extra Plasma Extra
Plasma/Whole Blood Biomarkers (Refer to Protocol)	Pink (K2 EDTA)	**6 ml**	1	Transfer 0.5 ml whole blood into **(4 cyrovials)**. Spin tube at 3000 × RPM/3.0 G for 10 mins, Collect plasma into**(4 cryovials)** Freeze remaining packed RBC	Ship Frozen[3], Store at -80 °C	Bio Markers 4 Whole Blood 4 plasma

[1]Ship in small box with gel packs and paper requisition. Write Questions or Concerns Please Call: Janelle Born 319-356-4797 Campus Mail: State Hygienics Lab – Coralville on box and also write final destination on box. Final Destination Shipping Address: Brian Wels PhD, Environmental Lab Scientist, State Hygienic Laboratory, 2220 S. Ankeny Blvd, Ankeny, IA 50023-6063, 515-725-1600 (Through Campus Mail, Drop off at First Floor Shipping). On the day specimens are shipped, e-mail Brian Wels at brian-wels@uiowa.edu of the pending specimen arrival.

[2]Ship to Eric (Rick) Devore, Wendy Hamilton (Location: 458 MRF) (wendy-hamilton@uiowa.edu), Donna 38140 (UI Campus). Record the amount of aliquots in lab log.

[3]Ship to Michael McCormick (Radiation Oncology) 335-8014, Antioxidant Core Lab (Location: B180 ML), Zita Sibenaller, Dr. Bryan Allen (UI Campus). Record the amount of aliquots in lab log.

- Purple K2E only drawn and processed on Day 1 and 14 of study.
- After collecting the plasma from the Pink (K2 EDTA 4-5 ml) tube, the packed RBC should be labeled with the following cycle, date drawn, patient study ID number then placed in the large SLM zip lock bag in freezer for batching monthly.
- All cryovials should have patients initials, ID number, cycle labs were drawn for, date of visit and the contents of the cryovials printed on them with sharpie.
- All cryovials should be frozen and stored with patients' information printed on them.
- Contents of cryovials should be plasma or whole blood.
- The EDTA 2 ml tube drawn for visit is not to be spun and taken to campus mail before 1330 to be shipped.

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20.0 SLM STUDY LAB CALENDAR ESCALATION SUBJECTS (BASED ON V9 PROTOCOL)

Test	Day 1	Day 14 (Cycle 1)	C2 (Day 1)	C3	C4	C5	C6	C7	C10	C13
SLM (3 ml purple tube)	Pre & Post 2 Hours	Pre & Post 2 Hours	Pre & Post 2 Hours	Pre & Post 2 Hours	Post 2 Hours	Pre & Post 2 Hours	Post 2 Hours	Pre & Post 2 Hours	Post 2 Hours	Post 2 Hours
miRNA (6ml purple tube)	Pre	Pre								
Biomarkers (6 ml pink tube)	Pre	Pre	Post 2 Hours	Post 2 Hours	Post 2 Hours	Post 2 Hours	Post 2 Hours	Post 2 Hours		

20.1 SLM STUDY LAB CALENDAR EXPANSION SUBJECTS

Test	D1	D14	C2	C3	C4	C7	C10	
SLM (3 ML purple tube)	Pre and 2 hour post	2 hr post only	2 hr post only	2 hr post only	2 hr post only	2 hr post only	2 hr post only	
miRNA (6 ml purple tube)	Pre	2 hr post only						
Biomarkers (6 ml pink tube)	Pre	2 hr post only	2 hr post only	2 hr post only	2 hr post only	2 hr post only	2 hr post only	

20.2 20.2 SLM STUDY LAB CALENDAR PILOT SUBJECTS

Test	D1	D14	C2	C3	C4	C7	C10	C13
SLM (3 ML purple tube)	Pre	2 hr post only	X	X	X	X	X	X
miRNA (6 ml purple tube)	Pre	2 hr post only						
Biomarkers (6 ml pink tube)	Pre	2 hr post only	X		X	X	X	X

21.0 DUAL ENERGY CT PROTOCOL

21.1 OBJECTIVE

From Dr. Zakharia: *The current plan is to do CT (your protocol) at baseline, day 14 (+/- 2 days) and at 2 months after SLM axitinib. All these 3 series will be done using your protocol. Dual Energy CT's will be covered by research money (day 14 scan does NOT need to be interpreted by Dr. Jinha Park).*

21.2 CT PROTOCOL

The same CT scan protocol will be obtained at 3 time points;

- Visit 1: baseline prior to treatment
- Visit 2: 14±2 days following start of SLM
- Visit 3: 56±3 days following start of chemotherapy (axitinib)

If for any reason baseline DECT could not be performed, we will utilize only regular CT scan subsequently in that setting (cancel further DECT) and there will NOT be a need for day 14 scan. This need to be discussed by PI before enrollment on study.

21.3 DUAL ENERGY CT:

- Contrast: 50% saline mixed with 50% 370mg/ml Iopamidol delivered at 4ml/s (4ml/s (35 s+ 5s) = 160ml of which 80ml is contrast) with delay of 35s (for chest) and 70s (for abdomen pelvis)
- Positioning: Cranialcaudal acquisition with scan coverage (DFOV) from pubic symphysis to apex of lungs (DFOV to match prior visit). Acquisition at coached total lung capacity (TLC) (recorded for DECT)
- Acquisition: CHEST: The spiral DECT contrast scan with dose modulation with tube A: 120 ref mAs/80kV, tube B: 67 ref mAs/Sn150 kV, with a pitch of 0.55 and rotation time of 0.25sec. The CTDIvol is assumed to be 4.28mGy, with total DLP of 143.2mGy*cm for a 30cm scan.
- Acquisition: ABDOMEN: The spiral dual energy contrast abdomen/pelvis scan protocol uses dose modulation with tube A: 190 ref mAs/100kV, tube B: 95 ref mAs/Sn150 kV, with a pitch of 0.55 and rotation time of 0.25sec. The CTDIvol is assumed to be 10.41mGy, with total DLP of 553.7mGy*cm for a 50cm scan.
- Reconstruction: R1: 0.75 x0.5; R2: 2.0 x 1.4, Qr40(Admire 5), FOV to match prior visits, Energy Series: A, B and M. Keep Raw data.

21.4 ANALYSIS

- Hypothesis: SLM will improve vascularization of tumors (ie increase in tumor iodine density at visit 2) which will allow for more effective chemotherapy treatment (ie decrease in tumor iodine density at visit 3, and decrease in tumor size). Secondary analysis may examine if baseline (visit 1) levels correlate with SLM response and/or treatment outcome (ie tumors with poor vascularization at visit 1, benefit more from SLM .

- Syngo.via (initial analysis on 2.0x 1.4 recon) to produce virtual non-contrast image (VNC), mono energetic 40keV, Iodine concentration PBV (HU and mg/ml)
- Examine analysis results using fixed region of interest (ROI) in tumor volume vs semi-automatic tumor segmentation.

21.5 COST

DECT data acquisition: 1hr scan charge ($470), supplies for injector etc ($25), contrast ($25), radiology read ($100) = $620 x 3 visits = $1,860 per subject
Analysis: Sieren grad RA, $25 x 4hr per case = $100

21.6 CRITERIA

- Tested and checked by HCCC nurse prior to each imaging study visit
  - Subject must have eGFR>45
  - Negative pregnancy test (women of child bearing age)
  - Schedule clinician available for attendance in CT facility during contrast administration

21.7 IRB CONSENT DOCUMENTATION

Catheter Placement and Blood Draw:
An IV catheter will be placed in your right arm by a Holden Comprehensive Cancer Center (HCCC) nurse or a study nurse coordinator. The catheter will be used during the perfused blood volume scanning procedures to deliver contrast agent or dye. Blood samples will also be drawn from the IV catheter to check your serum creatinine level (this test tells us how well your kidneys are functioning—if this level is abnormal, you will not be able to participate in the study). If you are a female of childbearing potential, you will have a urine pregnancy test on this return visit as well. The IV catheter will be kept in your arm for the duration of the study visit and will be used to deliver the contrast during the CT scan.

CT Scans:
You will then be taken to our CT Imaging Suite. Once there, the CT technologist will answer any questions you may have. If you have any metallic items on your body between your nose and abdomen (jewelry, zippers, piercings that can be easily removed, etc.) you will need to remove them prior to being put in the scanner. If you need to remove an article of clothing, you may use the private restroom in the CT Imaging Suite. Then you will be positioned on the moveable exam table of the CT scanner. We will connect you to an EKG machine (which will monitor your heart rate throughout the scans), a pulse oximeter (which is a small finger clip that has a sensor to monitor the amount of oxygen in your blood) and a blood pressure cuff. We will then move on to the scanning procedures:

For each visit a series of CT scans will be taken of your chest and you will be asked to hold your breath for about 5-8 seconds while the CT scans are taken.

- The first type of scans that will be done are called *Topograms.* These are a type of scout scans, which are similar to an x-ray, and help the CT technologist make sure that you are positioned correctly in the scanner. These are not counted as one of your CT scans. You will

be instructed to breathe normally and we will then tell you to take in as deep of a breath as you can and hold it while an image is being taken of your lungs. Once again, we will ask you to return to normal breathing for a few minutes.

- The *dual energy CT scan*: For these scans, a contrast agent (dye) will be injected through the IV catheter in your arm while the x-rays are taken. The injection of the contrast agent during the CT scanning allows us to see where and how blood distributes in your lungs. We will start the contrast injection and then ask you to take in a deep breath in and hold then have you hold your breath. The CT scanner is set to begin scanning your lungs after a certain amount of contrast has gone in. Once the scan is done, we will have you return to normal breathing.

21.8 SUPPORTIVE STUDIES ON WHICH THE PROTOCOL IS BUILT

Abdominal contrast timing (15-20s: early arterial, 35-40s: late arterial, 70-80s: late portal, 100s: nephrogenic phase).

From mRCC treatment response DECT published study (Hellbach et al. 2017):

- 1.3 ml/kg body weight 350mg/ml iomeprol at 2.5ml/s (~80ml contrast for 60kg adult) with delay of 70-80s (late portal phase for abdomen)
- Crainalcaudal from pubic symphysis to apex of lungs at TLC
- DECT protocol on Siemens 128-slcie with dose modulation (ref mAs not provided) tube A: 100kv, tube B: Sn140kV with pitch of 0.6 and rotation time of 0.5s

From NSCLC treatment response DECT published study (Baxa et al. 2016):

- 80mL 250mg/ml iomeprol at 4ml/s with 60ml saline flush (140ml of which 80ml contrast) at arterial bolus trigger (early arterial for abdomen) and bolus trigger +20s (late arterial phase for abdomen)
- DECT protocol on Siemens Flash with dose modulation tube A: 160 ref mAs/80kV, tube B: 68 ref mAs/Sn 140kV, pitch of 0.9 and rotation time of 0.33s. with average DLP of 149.6mGy*cm

From MESA study (current Hoffman study):

- 50% saline with 50% 370mg/ml Iopamidol at 4ml/s (4ml (17s+5s) = 96ml of which 48ml is contrast) with delay of 17s (early arterial phase for abdomen)
- Caudocranial at FRC
- DECT protocol on Siemens Force with dose modulation tube A: 210 ref mAs/80kV, tube B: 117 ref mAs/Sn150 kV, with a pitch of 0.55 and rotation time of 0.25sec. The CTDIvol is assumed to be 7.27mGy, with total DLP of 430mGy*cm for a 30cm scan.

From Smoking cessation (current Hoffman study):

- 50% saline with 50% 370mg/ml Iopamidol at 4ml/s with delay of 17s (early arterial phase - abdomen)
- Caudocranial at FRC
- The spiral DECT contrast scan protocol uses dose modulation with tube A: 120 ref mAs/80kV, tube B: 67 ref mAs/Sn150 kV, with a pitch of 0.55 and rotation time of 0.25sec. The CTDIvol is assumed to be 4.28mGy, with total DLP of 143.2mGy*cm for a 30cm scan.

21.9 REFERENCES

Baxa, J., T. Matouskova, G. Krakorova, B. Schmidt, T. Flohr, M. Sedlmair, J. Bejcek, and J. Ferda. 2016. 'Dual-Phase Dual-Energy CT in Patients Treated with Erlotinib for Advanced Non-Small Cell Lung Cancer: Possible Benefits of Iodine Quantification in Response Assessment', *Eur Radiol*, 26: 2828-36.

Hellbach, K., A. Sterzik, W. Sommer, M. Karpitschka, N. Hummel, J. Casuscelli, M. Ingrisch, M. Schlemmer, A. Graser, and M. Staehler. 2017. 'Dual energy CT allows for improved characterization of response to antiangiogenic treatment in patients with metastatic renal cell cancer', *Eur Radiol*, 27: 2532-37.