SARC Protocol #:	**SARC031**
TITLE:	A Phase 2 Trial of the MEK inhibitor selumetinib (AZD6244 hydrogen sulfate) in combination with the mTOR inhibitor sirolimus for patients with unresectable or metastatic malignant peripheral nerve sheath tumors
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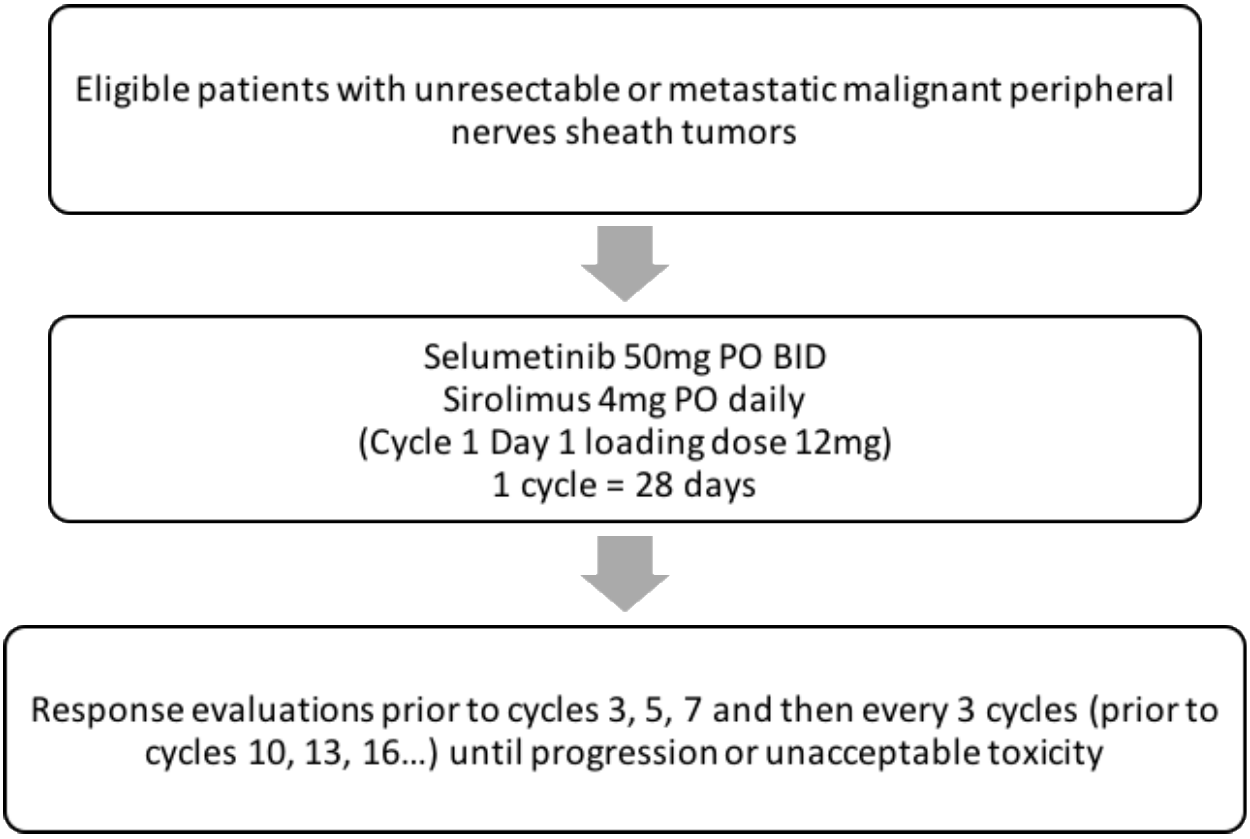
GLOSSARY OF ABBREVIATIONS

ADC	Apparent diffusion coefficient
AE	Adverse Event
ALP	Alkaline phosphatase
ALT (SGPT)	Alanine aminotransferase
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate aminotransferase
AUC	Area under the curve
CBR	Clinical benefit rate
CEC	Mature circulating endothelial cells
CEP	Circulating endothelial progenitor cells
CI	Confidence interval
CMR	Complete Metabolic Response
CR	Complete Response
CSR	Central serous retinopathy
CTC	Circulating Tumor Cells
DLT	Dose Limiting Toxicity
DoD	Department of Defense
DWI	Diffusion-weighted Imaging
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal growth factor receptor
EIAEDS	CYP3A4-Enzyme inducing anti-epileptic drugs
eIF2α	Eukaryotic translational initiation factor 2 alpha
ER	Endoplasmic reticulum
FISH	Fluorescence in situ hybridization
G6PD	Glucose 6-phosphate dehydrogenase
H-DLT	Hematological Dose-limiting toxicity
HCC	Hepatocellular Carcinoma
Hsp	Heat shock protein
IP	Intraperitoneal
LLN	Lower Limit of Normal
LVEF	Left Ventricular Ejection Fraction
MPNST	Malignant Peripheral Nerve Sheath Tumor

GLOSSARY OF ABBREVIATIONS

MRI	Magnetic Resonance Imaging
MUGA	Multiple Gated Acquisition scan
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Events
NF1	Neurofibromatosis Type 1
NH-DLT	Non-hematological Dose-limiting toxicity
OS	Overall Survival
ORR	Objective Response Rate
PMD	Progressive Metabolic Disease
PMR	Partial Metabolic Response
PBMC	Peripheral blood mononuclear cells
PD	Progressive disease or Pharmacodynamic
PERCIST	PET Response Criteria in Solid Tumors
PFS	Progression Free Survival
PS	Performance Status
PR	Partial Response
RECIST	Response Evaluation Criteria in Solid Tumors
RPED	Retinal pigment epithelial detachment
RVO	Retinal vein occlusion
SAE	Serious Adverse Event
SD	Stable Disease
SF	Shortening Fraction
SMD	Stable Metabolic Disease
TNM	primary tumor/regional lymph nodes/distant metastasis
TTP	Time to Tumor Progression

SCHEMA



SYNOPSIS

Primary Objective

To determine the clinical benefit rate of selumetinib in combination with sirolimus in patients with unresectable or metastatic NF1 associated or sporadic MPNST.

Secondary Objectives

- To define and describe the toxicities of selumetinib in combination with sirolimus in patients with unresectable or metastatic NF1 associated or sporadic MPNST.
- To assess the impact on pain intensity and pain interference and correlate to changes in clinical, imaging response and progression
- To assess progression free and overall survival

Exploratory Objectives

- To explore early FDG-PET and functional MRI changes to this combination and correlate to objective imaging response if feasible
- To evaluate changes in pharmacodynamic changes in pERK, pS6, pAKT, GLUT1 in blood and tumor tissue pre and post treatment in consenting patients with easily accessible tumors
- To explore circulating tumor DNA, germline and tumor genomic alterations in consenting patients with sporadic or NF1 associated MPNST
- To evaluate changes in immune infiltrates in blood and tumor tissue pre and post treatment

Hypothesis and Rationale

- Malignant peripheral nerve sheath tumors (MPNST) are the most common Neurofibromatosis Type 1 (NF1) associated malignancy and leading cause of death in patients with NF1
- Tumor regression in a transgenic MPNST mouse model occurred when inhibiting both mTORC1 and MEK, critical RAS effector pathways underlying the pathogenesis of NF1-mutant cancers
- The glucose transporter GLUT1 was potently suppressed only when both pathways were inhibited, which translated into early suppression in tumor uptake of FDG-PET in a dose dependent manner, and correlated with ultimate decrease in tumor size
- We hypothesize that selumetinib, a MEK inhibitor, in combination with sirolimus, an mTOR inhibitor, will cause tumor regression in patients with unresectable or refractory MPNST

Trial Design

- A Simon's two-stage phase 2 trial of MEK inhibitor selumetinib in combination with the mTOR inhibitor sirolimus to determine the safety and clinical benefit in patients with unresectable or metastatic MPNST

Clinical benefit rate will be defined as a CR, PR, or stable disease $\geq$ 4 cycles

Statistical Plan

Stage 1 will require 7 patients, with no further accrual if 0 of 7 respond. If 1 or greater of the 7 patients respond, accrual will continue until 21 patients have been enrolled. If 3 or greater of the 21 patients respond, this combination will be considered of sufficient activity, while 1-2 of 21 with benefit would be insufficient (Refer to section 13).

Maximum Total Number of Subjects

A total of 21 patients are expected to be enrolled. Stage 1 will require 7 patients and Stage 2 will require 14 patients.

Target Population

Individuals ≥ 12 years of age with unresectable or metastatic histologically confirmed sporadic or NF1 associated high grade MPNST who have experienced progression after one or more prior regimens of cytotoxic chemotherapy or for whom treatment on this protocol is felt to be in the best interest for patient in investigator's judgement.

Anticipated Length of Study

The anticipated accrual is 1-2 patients per quarter. Therefore, a maximum of 21 patients will be accrued over approximately 30 months. The median treatment duration per patient is estimated to be 6 months.

Study Drugs(s)

- Selumetinib (AZD6244) is an oral selective inhibitor of the mitogen-activated protein kinase (MEK) 1/2 currently in development for adult malignancies, pediatric low grade gliomas, and NF1 plexiform neurofibromas.

- Sirolimus is an oral mTOR inhibitor that has been FDA approved for immunosuppression following kidney transplantation.

Dosing and Administration

- Selumetinib will be given orally 50mg twice daily continuously and sirolimus will be given orally 4mg once daily with a cycle 1 day 1 loading dose of 12mg. One cycle will be 28 days.

- Patients will be able to remain on treatment as long as they do not experience progressive disease or unacceptable toxicity.

Efficacy Evaluations

- Response evaluation (RECIST) with appropriate imaging studies (MRI/CT) will be performed at baseline, prior to cycles 3, 5, 7 and then every 3 cycles (prior to cycles 10, 13, 16, etc.).

Safety Evaluations/Concerns

History and physical examinations and laboratory evaluations will be routinely performed during the study. Dose modifications and management plans are specified in the protocol.

Adverse event stopping boundary summary

- Stop when 3 patients experience grade 4 or higher non-hematologic adverse event if total number of patients accrued is 7 or less
- Stop when 1/3 of the patients experience grade 4 or higher non-hematologic adverse event if the total number of patients accrued is 8 or more

Mandatory Correlative Studies

- FDG-PET will be obtained at baseline and on Cycle 1 Day 11 (± 3 days) to assess for early FDG-PET response.
    - Imaging: For patients who have MRI imaging done for tumor disease evaluation, DWI/ADC mapping sequence will be performed if feasible at baseline and at the time of disease evaluation. This sequence will add less than 3 minutes of scanning time and will explore the value of functional MRI imaging technology.
- Pain assessments will be collected at baseline and prior to cycles 2, 3, 5, 9, 13 and then every 6 cycles to assess the impact on pain intensity and pain interference and correlate to changes in clinical, imaging response and progression.
- Blood samples will be collected at baseline and on Cycle 1 Day 15 (or once between Cycle 1 Day 15 through 28) to assess the following:
    - PBMCs will be assessed using flow cytometry for immune subsets.
    - Circulating endothelial progenitor cells (CEP) and mature circulating endothelial cells (CEC) will be assessed by flow cytometry.
    - Circulating Tumor Cells (CTCs) will be assessed using ferrofluidic enrichment and flow cytometry.
    - Evaluation of protein levels and MEK target inhibition.
    - Peripheral blood will be analyzed using the NanoString nCounter® platform to evaluate immune gene signature.
- ctDNA in peripheral blood at baseline and at the time of disease evaluation (prior to cycle 3, 5, 7 and then every 3 cycles).

Optional Correlative Studies

Tissue/tumor sample will be collected at baseline and on Cycle 1 Day 15 (or once between Cycle 1 Day 15 thru 28) for consenting patients with tumor safely accessible by percutaneous biopsy.

A minimum of two cores per biopsy are required for analysis and will be prioritized depending on number of cores obtained.

Tissue Correlatives (listed in order of priority based on tissue availability)

1. Analysis of immune gene signature using the NanoString nCounter® platform to analyze immune gene signature.
2. Evaluation of protein levels and MEK target inhibition.
3. Genomic and epigenetic profiling

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- To determine the clinical benefit rate of selumetinib in combination with sirolimus in patients with unresectable or metastatic NF1 associated or sporadic MPNST

1.2. Secondary Objective(s)

- To define and describe the toxicities of selumetinib in combination with sirolimus in patients with unresectable or metastatic NF1 associated or sporadic MPNST.
- To assess the impact on intensity and pain interference and correlate to changes in clinical, imaging response and progression
- To assess progression free and overall survival

1.3. Exploratory Objective(s)

- To explore early FDG-PET and functional MRI changes to this combination and correlate to objective imaging response if feasible
- To evaluate changes in pharmacodynamic changes in pERK, pS6, pAKT, GLUT1 in blood and tumor tissue pre and post treatment in patients with easily accessible tumors
- To explore circulating tumor DNA, germline and tumor genomic alterations in patients with sporadic or NF1 associated MPNST
- To evaluate changes in immune infiltrates in blood and tumor tissue pre and post treatment

2. BACKGROUND

2.1. Study Disease

2.1.1. Malignant Peripheral Nerve Sheath Tumors

Malignant peripheral nerve sheath tumors (MPNST) are aggressive soft tissue sarcomas associated with high risk of recurrence and metastasis [1]. MPNST account for 10% of all soft tissue sarcomas and carry the highest risk for sarcoma specific death among all the soft tissue histologies [2]. At present, complete surgical resection is the only curative treatment for MPNST, and conventional chemotherapy and radiation have not shown to reduce mortality in inoperable tumors [3-5] . The outcome for unresectable, recurrent, or metastatic MPNST is dismal.

2.1.2. Neurofibromatosis Type 1 and MPNST

Approximately half of all MPNST arise from individuals with Neurofibromatosis Type 1 (NF1) [6]. NF1 is a common autosomal dominant tumor predisposition syndrome. The NF1 gene encodes for a protein called neurofibromin, which

includes a GTPase activating protein that regulates hydrolysis of Ras-GTP to Ras-GDP [7, 8]. Patients with NF1 have dysfunctional or decreased levels of neurofibromin, which contributes to dysregulated Ras and tumorigenesis [9]. MPNSTs are the most common NF1 associated malignancy. The lifetime risk of MPNST in NF1 is 8-13% compared to 0.001% in the general population [6, 10]. The majority of NF1-associated MPNST arise from pre-existing plexiform neurofibromas [11], which may lead to delay in diagnosing MPNST in patients with NF1 because the clinical indicators of malignancy such as mass and pain may also be features of benign plexiform neurofibromas. NF1-associated MPNST in comparison to sporadic are more frequently located in the trunk, tend to be large (>5cm) [12] and may have greater propensity to metastasize [10]. These may be potential reasons why NF1-associated MPNST appear to have worse outcome than sporadic MPNST [13, 14]. Gene expression profiling of NF1 associated and sporadic MPNST did not identify molecular signatures that could reliably distinguish between both groups [15, 16].

2.1.3. Current Treatment for MPNST

The only known curative approach to MPNST is complete surgical resection with wide negative margins [3, 5]. Thus, the early diagnosis of MPNST is crucial, which may be difficult for NF1 patients for the reasons stated above. Adjuvant radiotherapy is recommended to improve local control in intermediate and high-risk lesions >5cm after marginal excision, but extremely high doses are needed and the local control rate is only 30-60% [17-19]. A recent prospective study evaluated the role of standard chemotherapy (doxorubicin, ifosfamide, and etoposide) in high grade unresectable MPNST (SARC006). Patients were stratified by sporadic vs. NF1-associated MPNST with the primary endpoint of determining objective response (CR or PR) after 4 cycles of chemotherapy. Local control with surgery and/or radiation followed with additional cycles of adjuvant chemotherapy. The objective response rate was lower in 17.2% in NF1 associated compared to 33% in sporadic, similar to what has been seen in retrospective reviews [3, 12] . In addition to objective responses, disease stabilization was achieved in most patients.

With increasing understanding in the molecular pathogenesis of MPNST, clinical trials of targeted agents have become available. Several histology specific trials with targeted agents have been performed for MPNST (Table 1). Although the trials that have been completed have not resulted activity in MPNST, they demonstrated that MPNST are aggressive cancers with rapid disease progression (median time to progression of <2 months) and that timely completion of trials for this rare malignancy is feasible.

Table 1. Completed and select ongoing clinical trials with targeted agents for refractory MPNST

Drug	Target	Phase	n =	Schedule	Outcome	Results	Ref
Erlotinib	EGFR	II	24	Oral continuous	Response WHO	19/20 pts. Progressive disease at 2 months 1 stable disease	[20]
Sorafenib	C-Raf, B-Raf, VEGFR2, C-Kit, PDGFR	II	12	Oral continuous	Response RECIST	No responses; median PFS 1.7 months	[21]
Imatinib	C-Kit, PDGFR, VEGFR	II	7	Oral continuous	Response RECIST	No responses; 1 stable disease	[22]
Dasatinib	C-Kit, SRC	II	14	Oral continuous	Response Choi	No response or stable disease	[23]
Alisertib	AURKA	II	10	Oral Continuous	Response RECIST	No response PFS 13 weeks	[24]
Bevacizumab/RAD001	Angiogenesis/ mTOR	II	25	IV q14d / oral continuous	Response WHO	No confirmed responses; 3 patients with stable disease $\geq$ 4 cycles	--
Ganetespib/ sirolimus	Hsp90 /mTOR	I/II	20	IV/Oral	WHO	1 patient with partial response	--

Based on high incidence of MPNST in NF1, limited treatment options, poor response to chemotherapy, high mortality, there is clearly a desperate need for more effective medical treatments for MPNST. Selection and prioritization of agents for clinical trials is a key challenge in drug development for NF1 as only a few agents can be tested in the clinical setting due to patient numbers, time, and cost. Transgenic mouse models of MPNST have become available, and preclinical trials in these models may have utility in the rational development of drugs for NF1 and MPNST.

2.1.4. mTORC1 and MEK inhibition in MPNST preclinical model

mTORC1 is a critical signaling component in NF1 mutant tumors; however, mTORC1 inhibition has only been shown to have cytostatic effects in MPNST models [25, 26]. Recently published data from the Cichowski laboratory demonstrated combining mTORC1 inhibition with a MEK inhibitor, which targets a second critical RAS effector pathway, induced tumor regression in a transgenic MPNST mouse model [27]. Performing transcriptional profiling and imaging studies, they identified GLUT1, which mediates 18F-fluorodeoxyglucose (18FDG) uptake, as a key gene that is suppressed prior to tumor regression, but only when BOTH pathways are effectively inhibited. Changes to the Glut1 mRNAs levels were detected after 14 hours of treatment, but GLUT1 protein was noted to be dramatically decreased after 72 hours of treatment suggesting repression is sustained. FDG-PET analysis in treated mice was performed 40 hours after baseline scan (to avoid any confounding change in FDG-PET signal due to reduction in tumor size). Mice treated with rapamycin in combination with 100%, 50%, and 25% of the MEK inhibitor produced a range of responses in FDG-PET uptake at 40 hours and tumor regression after 10 days and correlated in a dose dependent manner. These findings are suggestive that early changes in FDG-PET signal are indicative of target inhibition, which can correlate with

eventual tumor regression in MPNST treated with mTORC1/MEK inhibitors, and may have potential to serve as an imaging biomarker.

2.2. Study Agent(s)

2.2.1. Selumetinib (AZD6244)

Selumetinib (AZD6244) is an oral selective inhibitor of the mitogen-activated protein kinase (MEK) 1/2 currently in development for adult malignancies and pediatric low-grade gliomas. MEK is a critical kinase in the mitogen activated protein (MAP) kinase signal transduction pathway for many growth factor receptors that provide growth signals to cancer cells. It has been evaluated in a phase I trial in pediatric patients with NF1 and plexiform neurofibromas and thus far has been well tolerated over multiple cycles with promising initial results [28]. The adult recommended single agent dose is 75 mg BID on a continuous dosing schedule [29]. The most common adverse events (AEs) include acneform rash, diarrhea, nausea, vomiting, peripheral edema, oral mucositis, and dry skin. The recommended dose for selumetinib dose for children and young adults with low grade glioma and NF1 and plexiform neurofibromas is 25 mg/m^2/dose. Confirmed partial responses (plexiform neurofibroma volume decrease ≥20%) have been observed in 17 of 24 children and young adults in NF1 and plexiform neurofibromas suggesting that this dose inhibits pERK [28].

2.2.1.1. Preclinical Studies

The preclinical information provided in protocol is from the investigator's brochure and summarized here.

<u>In vitro studies</u>
These studies were done using the free-base of selumetinib.

The effect of selumetinib on ERK phosphorylation and cell viability was determined in a panel of 10 cells lines, including various tumor types (breast, colorectal, lung, osteosarcoma, ovarian, pancreatic, and prostate), in which the mutational status of BRAF and KRAS is known. The potency of selumetinib in inhibiting ERK1 and ERK2 phosphorylation was consistent among cell lines, ranging from 0.0018 to 0.0408μM.

<u>In vivo studies</u>
The pharmacological effects of selumetinib have been studied extensively by examining the anti-tumor activity of selumetinib in different tumors models including breast, melanoma, pancreatic, lung, and colon in mice which demonstrated strong anti-tumor activity in multiple models.

<u>Pharmacodynamic Markers in Tumor Samples</u>
The effects of single doses of selumetinib upon the levels of pERK in human Calu-6 xenograft tumors have been determined as a measure of selumetinib activity against MEK, and related to plasma levels of selumetinib. The levels of pERK in the tumor cell cytoplasm and nucleus were determined by

immunohistochemical (IHC) staining of formalin- fixed tissue sections, and western blot analysis of tumor protein lysates. Following an acute dose of 25 mg/kg selumetinib, pERK levels decreased by approximately 80% to 90% at 1, 2 and 4 hour post-dose, when plasma concentrations of selumetinib were highest, and recovered to >50% of control levels by 24 h. The same trend is observed by western blot analysis though the pERK signal was consistently, slightly higher than determined by IHC analysis.

Animal toxicities

Toxicities in preclinical studies, more frequent with chronic dosing, are not consistent through all animals. They included mineralization, defined as extracellular deposits of calcium and phosphate crystals, in multiple tissues, including cornea, kidney, liver, myocardium, skeletal muscle, diarrhea, renal changes (tubular epithelial swelling and mild vacuolation) secondary to persistent diarrhea, hematopoietic atrophy, and dilation of the corpus spongiosum, which lead to urethral compression.

2.2.1.2. Clinical Studies

Adult Phase 1

A Phase I, open label, multi-center study to assess the safety, tolerability and pharmacokinetics of selumetinib (capsules) in patients with advanced solid refractory malignancies for which no standard therapy exists has been completed. Efficacy assessment in this study was an exploratory endpoint.

The study was conducted in two parts. Part A (31 patients) of the study was a dose escalation study, was designed to provide adequate tolerability, safety, pharmacokinetic, and pharmacodynamic data. In Part A, the first cohort received a single 25 mg dose of the selumetinib capsule formulation of selumetinib on Day 1, followed by twice daily dosing from Day 2 onwards. Other doses investigated were 50 mg, 75 mg and 100 mg. The aim of Part B (29 patients randomized) was to determine the relative oral bioavailability of the selumetinib capsule, and secondly to expand the safety, tolerability and preliminary efficacy profile of the MTD from Part A (75mg BID). In Part B, patients received either a single dose of capsule formulation or free-base suspension formulation on Day 1 and 8, followed by continuous twice daily dosing of capsule formulation from Day 9 onwards.

Selumetinib was absorbed relatively quickly across all dose levels, with a median Tmax of 1.5 hours. Following the peak, AZD6244 concentrations declined multi-exponentially, with a mean T1/2 ranging from 5 to 7 hours, which is consistent across dose levels. CL/F and Vss/F also remained largely consistent across the dose range, with mean values ranging from 12 to 23 L/h and 87 to 126 L respectively. Plasma N-desmethyl selumetinib concentrations followed a similar pharmacokinetic profile to selumetinib, although exposure was much lower, with Cmax and AUC values generally <15% of parent, within each patient. The median Tmax was around 1.5 hours and T1/2 was around 9 to 13 hours. There was minimal accumulation after single versus multiple twice daily dosing. Limited data are available for

selumetinib amide. Concentrations of this metabolite are very variable, both between subjects and within the same subject on different study days. The time to maximum concentration was much more variable than either parent or the N- desmethyl metabolite, ranging from 0.5 to 24 hours across patients. Although there was insufficient data to calculate a terminal half-life, the available data suggest this is longer than that of either AZD6244 or N-desmethyl.

Dose-limiting toxicity (DLT) were grade 3 acneiform rash and pleural effusion. There was only 1 Grade 4 event in the study, an event of hypoglycemia. Fatigue (65.7%) and acneiform dermatitis (60.0%) were the most frequent adverse events at the MTD, with resolution of fatigue upon discontinuation of treatment. A patient with metastatic melanoma bearing a V600E BRAF mutation achieved a complete response persisting after 15 months of therapy.

Fifty-five patients had RECIST evaluable tumors. At the 75 mg dose 16/35 (45.7%) patients had stable disease for ≥6 weeks. One complete response was reported in a 30- year-old female patient with a BRAF mutation positive malignant melanoma receiving 75 mg twice daily of the selumetinib in Part A, which is ongoing at two years of therapy with selumetinib. Nine patients in Part A and 13 patients in Part B had a best response of stable disease. Ten out of 55 (18.2%) patients (not including the patient who had a CR) had stable disease of ≥16 weeks. Seven patients in Part A and 12 patients in Part B had a best response of progressive disease, and 10 patients in Part A and 3 patients in Part B were not evaluable for response. The MTD was 75mg BID [29].

Adult Phase II Studies

A Phase 2, double-blind, randomized study to assess the efficacy of selumetinib in combination with dacarbazine compared with dacarbazine alone in first line patients with BRAF mutation positive advanced cutaneous or unknown primary melanoma has been investigated [30]. Selumetinib 75mg or placebo is given twice daily in combination with dacarbazine. A total of 89 patients were randomized: 44 received selumetinib+dacarbazine and 45 received placebo+dacarbazine. There was statistically significant improvement in PFS (HR 0.63; 80% CI 0.47, 0.84; 1-sided p=0.021); median PFS was 5.6 months in the selumetinib+dacarbazine group compared with 3.0 months in the placebo+dacarbazine group.

A Phase 2, double-blind, randomized, placebo-controlled of selumetinib in combination with Docetaxel compared with Docetaxel alone, in second line patients with KRAS mutation positive locally advanced or metastatic non small cell lung cancer (Stage IIIB-IV) is completed [31]. Selumetinib 75mg or placebo is administered twice daily in combination with docetaxel 75 mg/m2 q21 days. A total of 86 patients were randomized with 44 receiving selumetinib and docetaxel and 45 with placebo and docetaxel.

The safety findings were generally as expected based on the monotherapy tolerability profiles of selumetinib and docetaxel. There was a statistically significant improvement in PFS (HR 0.58; 80% CI 0.42, 0.79; 1-sided p=0.014) with a median PFS of 5.3 months in the selumetinb+docetaxel group compared to placebo+docetaxel.

In an ongoing phase II trial of single agent selumetinib for adults with NF1 and inoperable plexiform neurofibromas (NCT02407405) selumetinib was initially administered at the adult recommended dose of 75 mg BID on a continuous doing schedule. The first 2 patients enrolled developed treatment limiting grade 3 acneiform rash associated with pain. This study was therefore amended to start all adult patients at a dose of 50 mg selumetinib BID with an option for dose increase if tolerated. The 50 mg BID dose has been tolerated well in this population, but further dose escalation has not been attempted in subsequently enrolled patients (B. Widemann, personal communication).[66]

Pediatric phase I trials:
In a pediatric phase I trial of selumetinib for children with low grade gliomas and in pediatric phase I trial for children and young adults with NF1 and inoperable plexiform neurofibromas the maximum tolerated dose was determined as 25 mg/m^2 BSA BID on a continuous dosing schedule. This dose corresponds approximately to a fixed adult dose of 50 mg BID, which will be used in the trial proposed here. [28]

2.2.2. Sirolimus

Sirolimus (rapamycin) is a mammalian target of rapamycin (mTOR) inhibitor that has been FDA approved for immunosuppression following kidney transplantation [32]. Sirolimus inhibits tumor growth in preclinical models by inducing cell cycle arrest and apoptosis, leading to recognition of the mTOR pathway as a target for cancer therapy [33, 34]. Rapamycin analogs, such as temsirolimus [35], an intravenous soluble ester (pro drug) of sirolimus and everolimus [36], and oral mTOR inhibitor, have been FDA approved for the treatment of advanced renal cell carcinoma. At this time, it is unclear whether one compound will have advantage over others in a particular tumor type.

Clinical Studies
A pharmacodynamic continuous reassessment method-based phase I study of rapamycin in adult patients with solid tumors was performed [37]. The pharmacodynamic endpoint used was skin phospho-P70 change after 28 days and effect was defined as at least 80% inhibition from baseline. Twenty-one patients enrolled at doses between 2 and 9 mg. Toxicities seen in at least 20% were hyperglycemia, hyperlipidemia, elevated transaminases, anemia, leucopenia, neutropenia, and mucositis. Hyperlipidemias responded well to statin treatments. The MTD was determined to be 6 mg daily on an uninterrupted schedule in solid cancer patients. Pharmacokinetics was similar to that seen in previous trials with

rapid absorption and slow elimination. Steady state was reached by day 8. There was an increase in day 28 half-life compared to day 1 (13 vs. 24 hours respectively). Five patients enrolled with previous progression on other therapy remained on drug for greater than 12 months.

Another phase I study of rapamycin was evaluated in advanced malignancies using a once weekly dose [38]. The MTD was determined to be 90 mg orally once weekly. The most common toxicities included nausea, diarrhea, asthenia, hyperglycemia, anemia, and lymphopenia. Preliminary evidence suggest that prolong suppression of phospho-S6K in peripheral T cells is possible at well-tolerated doses.

Sirolimus induced radiographic and clinical responses in three patients with malignant perivascular epithelioid cell tumors (PEComa), a rare tumor with no known previous treatments. Tuberous sclerosis (TSC) related tumors are characterized by constitutively activated mTOR signaling due to mutations in TSC1 and TSC2. Patients were treated to initially meet a target dose of 3-9 ng/mL and then 9-15 ng/mL after 16 weeks. Single agent sirolimus induced regression of tumors related to tuberous sclerosis with an overall response rate of 44% (16/36 had a partial response).

Sirolimus is being studied in combination with other agents for malignancy. A phase I study of sirolimus and bevacizumab in patients with advanced malignancies demonstrated that this combination was tolerable even when the drugs are combined at full doses [39]. Fatigue was the most common grade 3 toxicity. The recommended dose of sirolimus is 90 mg weekly (in two divided doses on consecutive days) or 4 mg daily in combination with bevacizumab 15 mg/kg IV q3weeks. Sirolimus has also been studied in combination with cytotoxic chemotherapy in refractory acute myelogenous leukemia [40] and chemoradiation in NSCLC [41] in phase I trials. In both studies, combination therapy was well tolerated. Sirolimus has been studied in combination with erlotinib in adults with recurrent glioblastoma (n=32). The doses of erlotinib and sirolimus were 150 mg and 5 mg for patients not on concurrent CYP3A-inducing anti-epileptics (EIAEDS), and 450 mg and 10 mg for patients on EIAEDS. The most common adverse effects (grade ≥ 2) were rash (59%), mucositis (34%), and diarrhea (31%). Grade 3 or higher events were rare.

In SARC023, a phase 1/2 combination study of ganetespib in combination with sirolimus for unresectable MPNST recently completed (manuscript in submission). The recommended dose of ganetespib was 200 mg/m^2 intravenously over one 1 hour on days 1, 8, 15 with sirolimus 4mg once daily continuous with a cycle 1 day 1 12mg loading dose. The mean (SD) sirolimus steady state trough at the recommended dose was 12.5 (8.9) ng/mL.

2.2.3. Combination of MEK and mTOR inhibitors in clinical trials

A phase II randomized study evaluating selumetinib versus selumetinib plus temsirolimus has been completed in adults with advanced soft tissue sarcoma (NCT01206140). Patients with advanced soft tissue sarcomas who received ≤ 2

prior chemotherapeutics were eligible, but pediatric type sarcomas, brain metastases were excluded, and MPNST patients were not enrolled on this trial. Sixty-nine subjects were randomized to selumetinib at 75 mg orally twice daily (n=34) versus selumetinib 50 mg orally twice daily (approximately 28 mg/m2/dose) and temsirolimus 20 mg IV once weekly (n=35) for 28 day cycles in patients with advanced soft tissue sarcomas. The combination arm was tolerated with acceptable side effect profile with most common grade 3/4 adverse events in the combination arm were mucositis (29%) and neutropenia and anemia (20%). There were no differences in progression free survival between the two cohorts. However, the combination does appear to be an active regimen in a subset of leiomyosarcomas with median improved progression free survival rates. Preclinical data suggesting activation of Akt-mTOR pathway in the development of leiomyosarcomas may contribute to this finding [42].

A phase 1 dose escalation study of selumetinib in combination with erlotinib or temsirolimus in patients with advanced solid tumors (no patients with MPNST enrolled) was also completed (NCT00600496) [43]. The MTD determined was temsirolimus 25mg once weekly with selumetinib 50mg BID with mucositis and neutropenia being dose limiting. The temsirolimus dosing is the recommended dose as monotherapy. The most common reported adverse events were nausea, fatigue, diarrhea, and mucositis. The PK profiles were comparable to previously observed monotherapy profiles.

A phase 1b study of trametinib and everolimus to determine the recommended phase 2 regimen was performed in patients with refractory solid tumors with planned expansion in pancreatic cancer [44]. Frequent treatment related adverse events were mucosal inflammation, stomatitis, fatigue, and diarrhea. Although the study evaluated multiple combinations with continuous and intermittent dosing, the study was unable to identify a recommended phase 2 dose that provided acceptable tolerability and adequate drug exposure.

A phase 1b study combining selumetinib and vistusertib in patients with advanced cancers (NCT02583542) was presented at the American Society of Clinical Oncology (ASCO) 2017 annual meeting in Chicago [45]. The study evaluated selumetinib 75mg orally twice daily continuously combined with 1) vistusertib orally daily continuously (CC dosing) and 2) vistusertib once daily intermittently (2 days on and 5 days off) (IC dosing). The most common ≥ Grade 3 adverse events (AE) were rash, fatigue, nausea, diarrhea, pain, anorexia, mucositis, and infection. The AEs were similar to the established single agent safety profiles. Two separate MTDs were defined for 1) continuous/continuous dosing which was 35mg BID vistusertib with 75mg BID selumetinib and 2) 125mg BID vistusertib 2 days on/5 days off with 75mg BID selumetinib. With regards to toxicity, the intermittent dosing schedule was preferable and was selected as the RP2D for the expansion phase 2 study. Recently the clinical development of vistusertib was discontinued by AstraZeneca due to lack of consistent target inhibition and activity.

2.3. Rationale

Based on strong preclinical rationale, we hypothesize that selumetinib in combination with sirolimus will cause tumor regression in patients with unresectable or metastatic MPNST. Sirolimus was selected for this trial due advantages of being an oral agent with well-known safety data; it was the agent used in the preclinical model, and we have pharmacokinetic data and inhibition data of dosing from our prior SARC023 data. Combination of selumetinib with temsirolimus (an intravenous prodrug of sirolimus) was found to be tolerable with acceptable side effect profile in two separate clinical trials {Infante, 2017 #76; Eroglu, 2014 #29}. FDG-PET imaging may be a potential imaging biomarker for target inhibition and response [27].

We propose a multi-institutional Simon's two-stage phase 2 study of selumetinib in combination with sirolimus for patients with unresectable or metastatic MPNST coordinated through SARC in collaboration with the NF Consortium.

2.4. Study Design

2.4.1. Overall Trial Design

A Simon's two-stage phase 2 trial of MEK inhibitor selumetinib in combination with the mTOR inhibitor sirolimus to determine the safety and clinical benefit in patients with unresectable or metastatic MPNSTs. Both agents will be given orally on an empty stomach. Selumetinib will be given orally at a dose of 50mg by mouth twice daily continuously. This is the recommended dose in combination studies of selumetinib with mTOR inhibition. Sirolimus will be given orally at a dose of 4mg by mouth once daily with a cycle 1 day 1 loading dose of 12mg. Each cycle will be considered 28 days. Refer to section 13 for statistical design.

FDG-PET will be obtained at baseline prior to therapy and on day 11 (+/-3 days) to assess for early FDG-PET response. Disease status will be evaluated using standard imaging techniques (CT/MRI) prior to cycles 3, 5, 7 and then every 3 cycles (prior to cycles 10, 13, 16, etc) using RECIST criteria.

All patients (i.e. inaccessible lesions) will participate in blood collection only prior to therapy and one day (ranging from day 15 to day 28) during cycle 1 for pharmacodynamics, genomic, and immune markers.

Patients who have MPNST that are accessible to percutaneous interventional radiological (IR) guided biopsy will have option to participate in tumor biopsy collection prior to therapy and one day (ranging from day 15 to day 28) during cycle 1 for the pharmacodynamics, genomic, and immune markers.
All patients on study will be closely monitored for the development of toxicity including regular physical examinations, laboratory evaluations, echocardiograms, and ophthalmology exams.

Patients will be able to remain on treatment as long as they do not experience progressive disease or unacceptable toxicity.

2.5. Correlative Studies Background

2.5.1. Imaging Pharmacodynamics

FDG-PET Scan

MPNST are FDG-PET avid and PET has been utilized to as radiologic method to potentially differentiate benign plexiform neurofibromas from MPNST. As described in section 2.1.3, FDG-PET may be indicative of target inhibition and may be predictive with eventual tumor regression, and thus be used as a potential imaging biomarker for tumor response in MPNST treated with mTOR/MEK inhibition. In this study, FDG-PET will be obtained (mandatory) at baseline prior to therapy and on day 11 (+/-3 days) to assess for early FDG-PET response. PET response will be evaluated per PERCIST guidelines [46] .

DWI/ADC Mapping with MRI

Data regarding the addition of functional MRI techniques may increase sensitivity and specificity of treatment response in soft tissue sarcomas [47-49]. The addition of DWI/ADC mapping adds less than 3 additional minutes of scan time, but may provide valuable information in this population. For patients who have MRI performed for disease staging and evaluation, DWI/ADC mapping sequence will be performed if feasible (mandatory if feasible) at baseline prior to therapy and during time of disease restaging (prior to cycle 3, 5, 7, and then every 3 cycles).

2.5.2. Plasma /Tissue Pharmacodynamics

Correlative studies evaluating pharmacodynamic parameters on MEK and mTOR inhibition will be explored in plasma and tumor tissue for consenting patients with tumors accessible safely by percutaneous biopsy (optional per consent). Phospho-S6 and pAKT are makers of choice in mTOR PD studies [37, 50]. pERK and 4EBPI will be evaluated for MEK target inhibition. Glut1 via Q-PCR and GLUT1 protein levels will be evaluated in blood and tumor tissue. Blood and tumor tissue will be taken at baseline prior to starting therapy and during cycle 1 on day 15 or after (i.e. one day from day 15 to day 28).

2.5.3. Immune Infiltrates and markers

Antitumor drugs including classical chemotherapeutics and molecular-targeted drugs have been shown to stimulate host antitumor immune responses [51, 52]. Recent clinical studies of immune checkpoint blockade in several cancer types have reported promising results with prolonged clinical responses and tolerable toxicity [52-54]. The role of the immune system in mediating anti-tumor responses in MPNST has not been explored in humans with MPNST to date.

Insights into the immunomodulatory impact of mTOR and MEK inhibition, which will be administered to patients with MPNST in the first clinical trial of this proposal, may offer opportunities to intelligently design combinatorial strategies to enhance antitumor immunity with the potential to produce durable partial or complete clinical responses in patients with MPNST.

All immune analyses described below will be performed under direction of **Jane Trepel (NCI, CCR)**, who leads the NCI, CCR preclinical development core. All

multiparameter flow analyses will be performed with a multiparametric flow cytometry (MACSQuant, Miltenyi Biotec, Bergisch Gladbach, DE), and data will be analyzed using FlowJo (FlowJo LLC, Ashland, OR) software. Serum and tumor tissue will be taken at baseline prior to starting therapy and during cycle 1 on day 15 or after (i.e. one day from day 15 to day 28)

Tumor Tissue analyses will be prioritized depending on the number of core biopsies that are obtained (optional per consent). One core biopsy will be required for analysis of the immune gene signature including tumor-associated antigen expression will be evaluated using the NanoString nCounter® platform (NanoString Technologies, Seattle, WA).

The following analyses will be performed from peripheral blood (mandatory) samples:

1) *Peripheral blood mononuclear cells (PBMC)* will be assessed using multiparameter flow cytometry for immune subsets including Tregs, MDSC, effector and exhausted CD4+ or CD8+ T-cells, and CD14+ monocytes. Assessment will include functional markers, i.e. PD1, PDL1, Tim3, CTLA4, HLA-DR and/or CD40.
2) *Circulating endothelial progenitor cells (CEP)* and mature circulating endothelial cells (CEC) will be assessed by multiparameter flow cytometry. Endothelial cells will be identified using co-expression of markers, including CD31, CD133 and CD146. The cell populations will also be analyzed for viability using scatter profiles and a vital stain, such as Hoechst 33258. Percentages of stained cells will be determined and compared with appropriate negative controls.
3) *Circulating Tumor Cells (CTCs):* Peripheral blood will be collected to correlate changes in CTCs with clinical response. CTCs will be assessed using ferrofluidic enrichment and multi-parameter flow cytometric detection.
4) Evaluation of the immune gene signature: Peripheral blood for analysis of the NanoString 770 gene Cancer Immune Panel will be collected in a PAXgene tube following manufacturer's instructions.

2.5.4. Genomic Profiling

Genomic abnormalities other than those at the NF1 locus are numerous in MPNST [15, 16] , which frequently arise in preexisting plexiform neurofibromas in NF1 patients [55]. For example, recurrent loss of *CDKN2A* was recently detected in atypical neurofibromas and MPNST but not in plexiform neurofibromas, supporting that atypical neurofibromas are precursor lesions for MPNST [55, 56]. Research focused on the molecular pathogenesis of MPNST has identified numerous potential targets, many of which are shared with cancers for which new drugs are being developed, and clinical trials for NF1 MPNST have become available [57-59]. The type of germline and somatic genomic alterations present in patients with MPNST may predict for response to treatment with targeted therapies, and potentially lead to identification of new targets for

treatment. As a first step towards the development of precision therapies we plan to comprehensively evaluate genomic alterations in patients with MPNST enrolled on this trial. Blood and tumor tissue (optional per consent) will be taken at baseline prior to starting therapy and tumor tissue during cycle 1 on day 15 or after (i.e. one day from day 15 to day 28).

We will perform comprehensive genomic and epigenetic profiling on the tumor samples in consenting patients using the matched normal for subtraction of germline variants. For genomic analysis, we will perform whole exome sequencing on tumor/matched normal and whole transcriptome sequencing on tumors using methods established by the NCI. For epigenetic profiling of tumors: to establish a baseline and evaluate whether gene expression and epigenetic landscape are altered by selumetinib and sirolimus we will perform Chip-seq of a) H3K27ac (enhancer regions), b) H3K27me3 (repressed genes), c) H3K4me1 (poised), d) H3K4me2 (actively transcribed and poised genes) d) H3K4me3 (poised and active), and CTCF (domain boundaries). We will also perform a recently described method of ATAC-seq to look for open chromatin regions [60]. For methylation we will utilize Illumina® Infinium™ HumanMethylation450 arrays. Sequencing will be performed in the Oncogenomics laboratory (**Dr. Shern/NCI**) using well-characterized commercial antibodies and the Illumina sequencing technology.

The comprehensive genomic analyses including next generation sequencing will be performed in the Shern laboratory **(Pediatric Oncology Branch/NCI).** Bioinformatics analysis will be primarily performed at the NCI using well-established pipelines developed at the NCI.

2.5.4.1. Management of Genetic Results

Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists). Clinically actionable findings will immediately be reported to SARC to notify the treating investigator so the treating investigator can verify result in a CLIA certified laboratory and provide genetic education and counseling. This is the only time during the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis. The generated genetic data will be made available in accordance with NCI Center for Cancer Research data sharing guidelines: (https://ccrod.cancer.gov/confluence.display/CCRGDS).

2.5.5. Circulating tumor DNA (ctDNA)

Sequencing circulating tumor (ct) DNA from patient plasma, also known as liquid biopsy, could lead to early quantification, serial tracking and potential correlative to disease burden and treatment response. The application of cell free DNA

technologies has yet to be systemically applied to patients with MPNST, and we propose to collect plasma samples from patients at baseline prior to therapy and then prior to each disease evaluation to explore whether ctDNA can be used to assess disease burden, treatment response, or progression.

2.5.6. Patient reported pain and impact of pain on daily activities (mandatory)

Pain associated with a mass was found to be the greatest risk factor associated with development of MPNSTs in NF1 [11]. Pain may also serve as a surrogate marker for tumor response and clinical benefit. We propose assessing patient reported pain severity and the impact of pain on daily activities prior to treatment and during treatment prior to cycles 2, 3, 5, 9, 13 and then every 6 cycles. We will explore the relationship of change in pain with radiologic response. The patient-reported pain evaluation will consist of two validated scales. The Numerical Rating Scale-11 (NRS-11) will be used to assess pain severity, and the Pain Interference Scale from the Patient Reported Outcomes Measurements Information System (PROMIS) will be will be used to assess the impact of pain on daily activities. These scales have been placed on a single page to simplify administration available in the operations manual. Total administration time is approximately 5 minutes. The patient reported pain severity and impact of pain on daily activities will reviewed and analyzed under the direction of **Dr. Pam Wolters and Staci Martin (NCI, CCR).**

i. The Numerical Rating Scale-11 (NRS-11) is a self-report segmented 11-point numeric scale that assesses pain severity [61]. It consists of a horizontal line with 0 representing "no pain" at the right end of the line and 10 representing "worst pain you can imagine" at the left end. Patients are asked to circle the one number from 0 to 10 that best describes how much their "most important tumor pain" hurt during the past week. It takes less than 1 minute to complete. The patient is instructed to rate the same area of tumor pain each time they complete the form so that changes from baseline can be examined.

ii. PROMIS Pain Interference Scale. The PROMIS-PI includes a self-report measure ($\geq$ 8 years) that assess the degree to which pain has interfered with the ability to complete daily activities over the past 7 days. There is a pediatric version for age 8-17 years and an adult version for ages 18 years and older. Items are rated on a 5-point Likert scale, and the measures yield standardized T-scores. It takes about 3-5 minutes to complete. This scale has demonstrated feasibility and validity in adults and youth with various medical conditions and chronic pain [62, 63] .

3. PATIENT SELECTION

3.1. Eligibility Criteria

Patients must have baseline evaluation performed prior to the first dose of study drugs and must meet all inclusion/exclusion criteria. Results of all baseline evaluations, which assure that all inclusion/exclusion criteria have been satisfied must be reviewed by the Principal Investigator or his/her designee prior to enrollment of the patient. In addition, the patient must be thoroughly informed about all aspects of the study, including study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

All clinical and laboratory studies must be performed within 14 days prior to enrollment unless otherwise indicated. Imaging studies for baseline scans must be obtained within 4 weeks prior to enrollment. Patients must start therapy no later than 7 calendar days after the date of study enrollment.

INFORMED CONSENT: All patients or guardians must sign a document of informed consent indicating their understanding of the investigational nature and the risks of this study before any protocol related studies are performed. Patients under 18 years of age will sign an assent document prior to treatment.

DURABLE POWER OF ATTORNEY (DPA): Patients will be offered the opportunity to assign a DPA so that another person can make decisions about their medical care if they become incapacitated or cognitively impaired.

3.1.1. AGE: ≥ 12 years of age

3.1.2. WEIGHT: ≥ 40 kg

3.1.3. DIAGNOSIS: Patients with unresectable or metastatic histologically confirmed sporadic or NF1 associated MPNST.

3.1.4. MEASURABLE DISEASE: Patients must have measureable disease by RECIST (see section11).

3.1.5. THERAPEUTIC OPTIONS: Patients must have experienced progression after one or more prior regimens of cytotoxic chemotherapy. Patients who have refused cytotoxic chemotherapy or for whom treatment on this protocol prior to receiving cytotoxic chemotherapy is felt to be in the best interest for the patient by the local investigator will also be eligible.

3.1.6. PRIOR THERAPY

- Patients must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering on this study.

- No limitation on the number of prior chemotherapy regimens that the patient may have received prior to study entry.

- Myelosuppressive chemotherapy: The last dose of all myelosuppressive anticancer drugs must be at least 3 weeks prior to study entry.

- Immunotherapy: The last dose of immunotherapy (monoclonal antibody or vaccine) must be at least 4 weeks prior to study entry.

- Biologic (anti-cancer agent): The last dose of all biologic agents for the treatment of the patient's cancer (such as retinoids or tyrosine kinase inhibitors) must be at least 7 days prior to study entry.

- Radiation therapy: The last dose of radiation to more than 25% of marrow containing bones (pelvis, spine, skull) must be at least 4 weeks prior to study entry. The last dose of all other local palliative (limited port) radiation must be at least 2 weeks prior to study entry.

- Stem Cell Transplantation. At least 2 months post-autologous stem cell transplant or at least 3 months post-allogeneic transplant and recovered from toxicities without evidence of graft versus host disease and on stable doses of immunosuppressive medications if required.

- Growth Factors. The last dose of colony stimulating factors, such as filgrastim, sargramostim, and erythropoietin, must be at least 1 week prior to study entry, the last dose of long-acting colony stimulating factors, such as pegfilgrastim, must be at least 2 weeks prior to study entry.

3.1.7. CONCURRENT THERAPIES: No other anti-cancer therapy (chemotherapy, biological therapy, radiation therapy) is permitted.

3.1.8. PERFORMANCE STATUS

- Lansky/Karnofsky performance level ≥ 50% (See Appendix I).

- Patients who are unable to walk because of paralysis or motor weakness, but who are up in a wheelchair will be considered ambulatory for the purpose of calculating the performance score.

3.1.9. HEMATOLOGIC FUNCTION

- Peripheral absolute neutrophil count (ANC) of ≥1000/μL

- Platelet count ≥75,000/μL (transfusion independent (no transfusion within at least 7 days prior to enrollment))

3.1.10. HEPATIC FUNCTION

- Total bilirubin must be ≤ 1.5 times the upper limit of normal (ULN)

- SGPT (ALT) must be ≤ 3.0 times ULN

3.1.11. RENAL FUNCTION: Serum creatinine ≤ ULN or creatinine clearance >60 ml/min/1.73 m2

3.1.12. Serum triglyceride level ≤300 mg/dL and serum cholesterol level ≤ 300 mg/dL (Patient may be on lipid-lowering medicine)

3.1.13. CARDIAC FUNCTION:

3.1.13.1. Normal ejection fraction by ECHO or cardiac MRI >55%

3.1.13.2. QTcF ≤ 450ms

3.1.14. Fertile men and women of childbearing potential must agree to use an effective method of birth control.

Female patients of child bearing potential must be willing to use 2 forms of contraception (per institutional standards) from the time of screening until 4 weeks after discontinuing the study (1 highly effective and 1 barrier method, see below). They must not be breastfeeding and must have negative pregnancy test prior to start of dosing.

For a female patient to be considered as of not child bearing potential, she should fulfil one of the following:

- Post menopausal women, defined as either women aged more than 50 years and have amenorrhea for at least 12 months following cessation of all exogenous hormonal treatments, or, women under 50 years who have amenorrhea for at least 12 months following cessation of exogenous hormonal treatments, and have serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels in the postmenopausal range for the institution.

OR

- Have documentation of irreversible surgical sterilization by hysterectomy, bilateral oophorectomy or bilateral salpingectomy (but not tubal ligation)

- Have medically confirmed, irreversible premature ovarian failure.

Highly effective methods of contraception are:

- Use of medroxyprogesterone acetate depot injection (Depo-provera™). (Please note: use of any other oral, injected, or implanted hormonal methods of contraception cannot be considered highly

effective as it is currently unknown whether selumetinib/sirolimus may reduce their effectiveness)

- Placement of a copper-banded intrauterine device (IUD) or intrauterine system (IUS)
- True abstinence
- Bilateral tubal ligation
- Vasectomized partner

Barrier methods include:

- Condom
- Occlusive cap (e.g. diaphragm or cervical/vault caps) with spermicide

Male patients should either be surgically sterile or willing to use an effective barrier method of contraception during the study and for 16 weeks following the last dose of study treatment if sexually active with a female of child bearing potential. If not done, storage of sperm prior to receiving study treatment will be advised to male patients with a desire to have children.

3.1.15. Patients with central nervous system disease are eligible for enrollment if they have received prior radiotherapy or surgery to sites of CNS metastatic disease and are without evidence of clinical progression or stable disease at 4 weeks.

3.2. Exclusion Criteria

3.2.1. Patients receiving other anti-cancer agents are not eligible.

3.2.2. Patients who cannot swallow whole pills.

3.2.3. Patients receiving chronic, systemic treatment with corticosteroids or another immunosuppressive agent (for example cyclosporine). Topical or inhaled corticosteroids are allowed.

3.2.4. Patients should not receive immunizations with attenuated live vaccines within four weeks of study entry or during study period.

3.2.5. Any recent major surgery within a minimum of 4 weeks, with the exception of surgical placement for vascular access, or minor surgery (excluding tumor biopsies) within 14 days.

3.2.6. Patients who any known severe and/or uncontrolled medical conditions or other conditions that could affect their participation in the study such as:

- Severely impaired lung function defined as spirometry and DLCO that is 50% of the normal predicted value corrected for hemoglobin and alveolar volume and/or O2 saturation that is 88% or less at rest on room air. For patients who do NOT have respiratory symptoms (e.g. dyspnea at rest, known requirement for supplemental oxygen), pulmonary function test is not required.

- Cardiac conditions as follows:

    - Uncontrolled hypertension (blood pressure ≥150/95 mmHg despite medical therapy.

    - Acute coronary syndrome within 6 months prior to starting treatment

    - Uncontrolled angina despite medical therapy

    - Symptomatic heart failure NYHA Class II-IV prior or current cardiomyopathy, or severe valvular disease

    - Prior or current cardiomyopathy

- Uncontrolled Type 1 or 2 diabetes as defined by fasting serum glucose >1.5 x ULN

- Uncontrolled infection

- Pre-existing renal disease including glomerulonephritis, nephritic syndrome, Fanconi Syndrome, or renal tubular acidosis.

- Current refractory nausea and vomiting, malabsorption syndrome, disease significantly affecting gastrointestinal function, resection of small bowel, symptomatic inflammatory bowel disease, or ulcerative colitis, or partial or complete bowel obstruction.

- Ophthalmological conditions as follows:

    - Current or past history of retinal pigment epithelial detachment (RPED)/central serous retinopathy (CSR) or retinal vein occlusion

    - Intraocular pressure (IOP) > 21 mmHg or uncontrolled glaucoma

3.2.7. Supplementation with vitamin E greater than 100% of the daily recommended dose.

3.2.8. Hypersensitivity to active or inactive excipients of rapamycins (sirolimus, temsirolimus or everolimus) or selumetinib or drugs with similar chemical structures or class to sirolimus or selumetinib.

3.2.9. Patients unwilling or unable to comply with the protocol.

3.2.10. Seville orange, star fruit, grapefruit and their juices, and St. John's Wort use are not allowed while on study.

3.2.11. Exposure to strong or moderate inhibitors or inducers of CYP3A4/5, Pgp (MDR1) and BCRP if taken within the stated washout periods before the first dose of study treatment (Appendix II).

3.2.12. Exposure to specific substrates of drug transporters OATP1B1, OATP1B3, MATE1 and MATE2K within the appropriate washout periods (a minimum of 5 x reported elimination half-life) before the first dose of study treatment (Appendix II)

3.3. Inclusion of Children, Women and Minorities

Men, women, children (as applicable) and members of all ethnic and racial groups are eligible for this trial.

Subjects of both genders and from all racial and ethnic groups are eligible for this trial if they meet the eligibility criteria outlined in Section 3.1. No groups are being excluded from participation in the trial. Approximately 50% of MPNST develop in individuals with NF1, and we expect that approximately 50% of individuals enrolled will have NF1 associated MPNST and 50% will have sporadic MPNST.

The treatment approach to MPNST is similar for children and adults. Approved adult and adolescent drug dosing was determined to be equivalent in approximately 95% of all products with an adolescent indication studied under the FDA amendments act of 2007 [64]. Whereas sporadic MPNST are typically diagnosed in late adulthood, MPNST diagnosis peaks at a younger age in patients with NF1, generally in early adulthood (20-50 years) with 10-20% of cases reported at event younger ages (1-19 years) [12, 13]. Thus, treating patients ≥ 12 years of age will allow for the majority of patients with MPNST without affecting safety and dosing per FDA findings.

3.4. Human Subjects Protection

3.4.1. Risks/Benefits Analysis

The primary risk to patients participating in this research study is from toxicity of the combination of selumetinib and sirolimus. The primary objective of this trial is to determine the clinical response rate in patients with refractory MPNST. Safety and tolerability will be closely monitored. Patients will thus be treated with therapeutic intent and response to the therapy will be closely monitored. Treatment options for these patients are very limited, as most patients will have received prior cytotoxic chemotherapy, which is often considered first line treatment by many oncologists for unresectable high-grade MPNSTs. The protocol provides for detailed and careful monitoring of all patients to assess for toxicity and response to treatment. Patients will be treated with therapeutic intent and response to the therapy will be closely monitored. The potential benefit from this therapy is disease stabilization, tumor shrinkage, and decrease tumor related

symptoms. Therefore, this protocol involves greater than minimal risk to subjects, but presents the potential for direct benefit to individual subjects. For patients who cannot provide informed consent by themselves, but have a durable power of attorney (DPA) this legal representative will be able to provide informed consent for this study.

The medical, hospital, and research records associated with this study are considered confidential. Members of the treating team and designated research study assistants will have access to the records as required to administer treatment and comply with the protocol. Neither the name nor other identifying information for an individual will be used in the report or publication concerning this study. Patient records may be inspected by auditing agencies including the DoD, FDA, and Drug companies that make selumetinib and sirolimus.

3.4.2. Informed Consent

The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and benefits, and potential alternative therapies will be carefully explained to the patient or the patient's parents or guardian if the patient is a child, and a signed informed consent and assent document will be obtained according to institutional guidelines prior to entry onto the study. Consent will be obtained by the PI or an associate investigator on this trial. This must be done according to the guidelines provided in the Declaration of Helsinki, ICH E6 Guideline for Good Clinical Practice (GCP), and requirements of Title 21 CFR 50.20 through 50.27. The patient must be made aware and agree that personal information may be scrutinized during audit by competent authorities and properly authorized persons. However, personal information will be treated as strictly confidential and will not be publicly available.

4. REGISTRATION PROCEDURES

4.1. General Guidelines

After obtaining Informed Consent, eligible patients will be enrolled on this trial. Subjects will be registered by local sites through an electronic database, and will be issued a subject unique identifying numbers for eligible participants. An investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each subject treated with the investigational product in the study or registered to the study. SARC may request faxed copies of selected source documents with PHI redacted for verification of records, accuracy of electronic submissions and review of data.

While all study evaluations must be performed by the Investigator as described in Section 10, Study Evaluations and Study Calendar, only data related to the primary and secondary endpoints, as well as safety data, will be captured in the eCRFs.

4.2. Registration Process

This study uses a web based data entry system for data submission. All subject registrations and Case Report Forms (CRFs) will be submitted electronically via the study web site. All subjects must be registered on the study website prior to start of treatment. Data Managers and other authorized users will be provided with a unique user identification number and password to access the site. All study case report forms may be accessed online through the study website. In case there are problems accessing the website, please contact the SARC office directly at: Phone: 734-930-7600, Fax: 734-930-7557.

5. TREATMENT PLAN

5.1. Agent Administration

Treatment Schedule		
Selumetinib (mg)	**Sirolimus (mg)**	
PO BID continuous	**Cycle 1 Day 1 loading dose**	**Maintenance PO once daily continuous**
50	**12**	**4**
1 cycle = 28 days		

5.1.1. Selumetinib

Selumetinib will be supplied in 10mg and 25mg capsules. Dosing will be orally 50mg twice daily continuously. Patients should be instructed to take selumetinib on an empty stomach (either 1 hour before or 2 hours after meals) with water only. If a patient misses a dose, the dose may be taken within 4 hours of missed dose, otherwise wait until the next dose. If a patient vomits after a dose, it will not be repeated.

The capsules cannot be crushed and have to be swallowed whole. Patients will keep a diary (see operations manual) to document the intake of each dose of selumetinib and potential side effects. The patient diary review and pill count will be done prior to each cycle.

5.1.2. Sirolimus

Sirolimus will be purchased commercially (2mg tablets) and administered once daily. On cycle 1 on day 1, a loading dose of sirolimus will be given orally x 1. Subsequent doses of sirolimus will be 4mg given orally once daily continuously with no breaks in between cycles. Sirolimus should be taken around the same time as the morning dose of selumetinib. Sirolimus should be taken at approximately the same time every day. If a patient misses a dose, the dose may be taken within 4 hours of missed dose. Otherwise, patient must wait until the next day's dose. If a patient vomits after a dose of sirolimus it will not be repeated. Tablets must be swallowed whole. All doses prescribed and dispensed to the patient and all dose changes during the study will be recorded in the patient diary.

One cycle of therapy is 28 days.

5.2. Criteria to start subsequent cycles

(Refer to section 6 for dose modifications)

A cycle may be repeated every 28 days if the patient has at least stable disease, has again met the ANC, platelet, hepatic, and renal function defined in Section 3.1, and has not met one of the off protocol therapy or off study criteria in Section 5.6 and 5.8. Up to 21 days (to day 49) will be allowed between cycles for recovery without modifying drugs on subsequent cycles if toxicity did not meet criteria requiring dose modification.

5.3. Duration of treatment

Patients may continue therapy as long as they achieve a minimum response of stable disease and do not experience excessive toxicity.

5.4. General Concomitant Medication and Supportive Care Guidelines

5.4.1. Concurrent Therapy

- Concurrent cancer therapy including chemotherapy, radiation therapy, immunotherapy or biologic therapy may NOT be administered to patients while on this study. If these treatments are administered, the patient will be removed from protocol therapy.

- No other investigational agents may be given while the patient is on study.

5.4.2. Concomitant Medications

- Doses of vitamin E greater than 100% of daily recommended doses are contraindicated.

- Throughout the study, patients should be instructed to avoid changes to or the addition of concomitant medications, in particular any that may affect the metabolism of selumetinib and sirolimus, unless considered clinically indicated.

- While a patient may not enter a study arm if they have taken within the stated washout periods prior to study start any of the CYP450 isoenzyme inhibitors or inducers detailed in exclusion criteria and in Appendix II, it could be possible to allow their short-term administration during the study if clinically indicated and unavoidable.

5.4.3. Supportive care

Appropriate antibiotics, blood products, anti-emetics, fluids, electrolytes, and general supportive care are to be used as necessary.

5.4.4. Skin and sunlight protection

Recommendations to start on day one of treatment with selumetinib and sirolimus and to continue throughout treatment and 3 months post treatment.

- Use skin moisturizer (thick, alcohol free) at bedtime
- Avoid excessive exposure to sunlight
- Use sunglasses and sunscreen (PABA-free, SPF ≥15; UVA and UVB protection) as needed
- Use of topical retinoids or benzoyl peroxide is not recommended

5.4.5. Management of Rash

Early identification and intervention is critical for optimal management of rash. Patients who develop Grade 1 changes, consider anti-histaminergic drugs, mild/moderate topical steroid and/or topical antibiotic. For Grade 2 changes, consider anti-histaminergic drugs, a moderate strength topical steroid and consider oral antibiotics.

For Grade ≥ 3 rash, interrupt selumetinib/sirolimus. Apply moderate strength topical steroid and consider an oral antibiotic. If an infection is suspected, consider other broad spectrum antibiotic coverage. If rash has improved to a Grade 2 or less, restart selumetinib/sirolimus at reduced dose.

Examples of treatments are:

- Topical of moderate strength: triamcinolone, desonide, aclometasone
- Oral antihistamines: loratidine, cetrizine, fexofenadine, diphenhydramine, hydroxyzine

- Topical antibiotics: clindamycin, erythromycin, metronidazole, silver sulphadiazine
- Oral antibiotics: doxycycline, minocycline, oxytetracycline

5.4.6. Management of stomatitis/oral mucositis/mouth ulcers

For mild toxicity (Grade 1), use conservative measures such as non-alcoholic mouth wash or salt water (0.9%) mouth wash several times immediately after drug administration (1 to 3 hours) and during the day as required until resolution. For more severe toxicity (Grade 2 or 3), the suggested treatments are topical analgesic mouth treatments (i.e. local anesthetics such as benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol, or phenolic compounds) with or without topical corticosteroids such as triamcinolone oral paste 0.1% (e.g. Kenalog in Orabase®) or alcohol-free 0.5 to 2mg/5mL dexamethasone oral solution (i.e. for example Dexsol® or PMS dexamethasone 0.5mg/5mL elixir). The mouth rinse will be self-administered at a daily dose of 10mL per day. Most importantly, patients must be instructed to swish and expectorate the mouth rinse to avoid systemic exposure to dexamethasone. Agents containing hydrogen peroxide, iodine, and thyme derivatives may worsen mouth ulcers. It is preferable to avoid these agents. For Grade $\geq$ 3 stomatitis/oral mucositis/mouth ulcers, systemic pain killers are indicated.

5.4.7. Management of diarrhea

Patients should be aware diarrhea can occur with selumetinib/sirolimus treatment. Patients should be given loperamide (in accordance with local practice) to take home with them and advised to start after first episode of unformed stool. Patients should be given dietary advice in case of diarrhea (e.g. BRAT [bananas, rice, apple sauce, toast, plain past] diet; readily digestible food, avoidance of lactose-containing products, fried, fatty or spicy food) and increase fluid intake (8-10 glasses of clear fluid daily, including water and fluids containing salt and sugar such as sports drinks and clear broth).

For uncomplicated Grade 1 or 2 diarrhea:

- Patients should immediately start loperamide after the first episode of diarrhea (4mg initially) and continue (2mg every 4 hours or after each unformed stool) until they have been free for at least 12 hours.
- If after 12 hours of loperamide treatment, the diarrhea is not improving, can increase to high dose loperamide (2mg every 2 hours or 4mg every 4 hours) and continue to take loperamide until they have been free from diarrhea for at least 12 hours. Additional treatment may be considered according to local practice.

**NOTE: Should NOT exceed 16mg loperamide (i.e. 8 capsules/tablets) over a 24 hour period

For persistent (>24hours) Grade 1 or 2 diarrhea despite loperamide at high dose, the following should be considered:

- Rehydration and electrolyte replacement as appropriate
- Infectious causes and etiologies such as clostridium difficile or viral enteritis
- Antibiotics if appropriate particularly if patient is neutropenic or has fever
- Discontinuation of loperamide and start of octerotide (sandostatin)
- Addition of other second line anti-diarrheal agents according to local practice
- Hospitalization

For any Grade uncontrolled or complicated diarrhea or Grade 3-4 diarrhea, consider:

- Hospitalization
- Intravenous fluids, electrolytes, and antibiotics if needed
- Start octreotide (sandostatin)

5.4.8. Management of hyperlipidemia and hyperglycemia

Treatment of hyperlipidemia and hyperglycemia should consider the pre-treatment status and dietary habits. Blood tests to monitor hyperlipidemia must be taken in the fasting state. Grade ≥2 hypercholesterolemia or hypertriglyceridemia should be treated with a 3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase inhibitor (e.g. atorvastatin, pravastatin) or appropriate lipid-lowering medication, in addition to diet. Patients should be monitored clinically and through serum biochemistry for the development of rhabdomyolysis and other adverse events as required in the product label/data sheets for HMG-CoA reductase inhibitors.

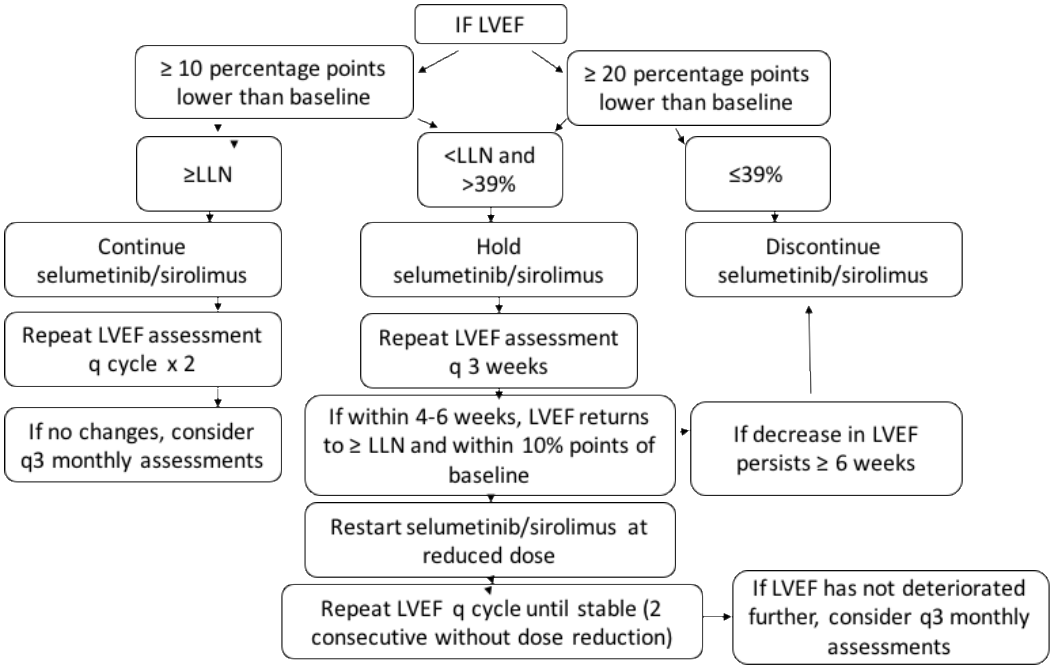
5.4.9. Management of non-infectious pneumonitis

Non-infectious pneumonitis is a class effect of rapamycin derivatives. Cases of non-infectious pneumonitis (including interstitial lung disease) have also been described. A diagnosis of non-infectious pneumonitis should be considered in patients presenting with non-specific respiratory signs and symptoms such as hypoxia, pleural effusion, cough, or dyspnea and in whom infectious, neoplastic, or other non-medicinal causes have been excluded by means of appropriate investigations. Patients should be advised to report promptly any new or worsening respiratory symptoms. For patients who develop radiological changes suggestive of non-infectious pneumonitis, and have few or no symptoms, may continue sirolimus with dose alteration. Dose modifications and retreatment are described in table below:

Worst Grade Pneumonitis	**Required Investigations**	**Management of Pneumonitis**	**Sirolimus Dose Adjustment**
Grade 1	CT scans with lung windows and pulmonary function testing including: spirometry, DLCO, and room air O_2 saturation at rest. Repeat chest x-ray/CT scan every 2 cycles until return to baseline.	No specific therapy is required	No change in dose.
Grade 2	CT scan with lung windows and pulmonary function testing including: spirometry, DLCO, and room air O_2 saturation at rest. Repeat each subsequent cycle until return to baseline. Consider bronchoscopy *	Symptomatic only. Prescribe corticosteroids if cough is troublesome.	Reduce sirolimus dose to 50% lower dose than previously administered until recovery to ≤ Grade 1. Sirolimus may also be interrupted if symptoms are troublesome. Patients will be withdrawn from protocol treatment if they fail to recover to ≤ Grade 1 within 3 weeks.
Grade 3	CT scan with lung windows and pulmonary function testing including: spirometry, DLCO, and room air O_2 saturation at rest; Repeat each subsequent cycle until return to baseline. Bronchoscopy is recommended *	Prescribe corticosteroids if infective origin is ruled out. Taper as medically indicated.	Hold treatment until recovery to ≤ Grade 1. May restart protocol treatment within 2 weeks at a reduced dose if evidence of clinical benefit. Patients will be withdrawn from the study if they fail to recover to ≤ Grade 1 within 2 weeks.
Grade 4	CT scan with lung windows and required pulmonary function testing includes: spirometry, DLCO, and room air O_2 saturation at rest. Repeat each subsequent cycle until return to baseline. Bronchoscopy is recommended *.	Prescribe corticosteroids if infective origin is ruled out. Taper as medically indicated.	Discontinue treatment.

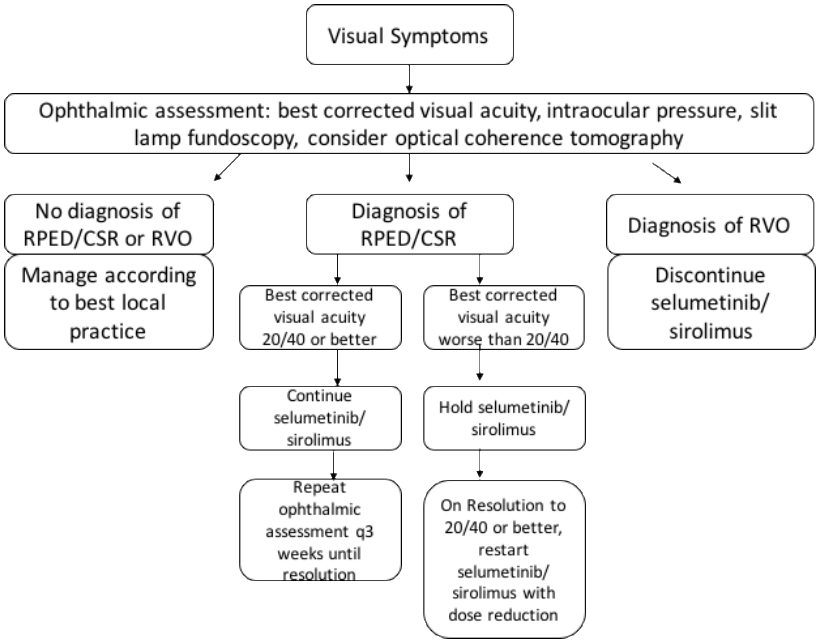
***A bronchoscopy with biopsy and/or bronchoalveolar lavage should be considered (grade 2) or is recommended (grade 3 or 4). For any grade infection should be ruled out prior to prescribing corticosteroids.**

5.4.10. Management of asymptomatic left ventricular ejection fraction (LVEF) reduction



Abbreviations: LVEF, Left ventricular ejection fraction; LLN, lower limits of normal

5.4.11. Management of patients with visual symptoms or abnormal findings



Abbreviations: RPED, retinal pigment epithelial detachment; CSR; central serous retinopathy; RVO, retinal vein occlusion

5.5. Criteria for Removal off Protocol Therapy

Treatment may continue until one of the following criteria applies:

- Progressive Disease
  - Patients with radiographic or clinical evidence of disease progression following any treatment cycle will discontinue protocol therapy.

- Toxicity
  - Adverse events requiring removal from therapy.
  - Patient has not met parameter to start subsequent cycle.

- Administrative
  - Patient refusal of further treatments. Reasons must be noted on the patient's record.
  - If deemed in the best interest of the patient to be taken off protocol therapy, the Principal Investigator should be notified and the reasons for withdrawal noted in the patient's medical record.
  - Serious protocol violation as determined by the Principal Investigator
  -
- Another Medical
  - Development of any concurrent medical condition that precludes further administration of therapy.

5.6. Duration of Follow Up

Patients will be followed for adverse event(s) until 30 days after last treatment or until resolution or stabilization of the adverse event (whichever comes last). Patients who are removed from protocol therapy for a reason other than disease progression, should be followed up every 6 months by imaging to monitor disease status. Every effort should be made to collect information regarding disease status until the start of new anti-cancer therapy, disease progression, or death. Once a patient experiences confirmed disease progression or starts a new anti-cancer therapy, the patient should be contacted by telephone every 6 months to assess for survival status until death or withdrawal of consent or end of study, whichever occurs first.

5.7. Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed below applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form including death or lost to follow up.

- Patient or guardian withdrawal of consent. Reasons must be noted on the patient's record.

- Death

- Lost to follow up

- Enrollment onto another SARC treatment study or SARC long term follow up study.

6. DOSING DELAYS / DOSE MODIFICATIONS

Patients who experience a toxicity requiring dose modification attributed to selumetinib and sirolimus but in the opinion of the investigator have benefited from the prior treatment cycle (decrease in size or stabilization of tumor or decrease in tumor related symptoms such as pain) may continue on study at lower doses of causative agent. This study will use CTEP Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 for toxicity and adverse event reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov). If toxicity cannot be clearly attributed to either agent alone, the toxicity will be attributed to both agents and modifications will be made to both agents. Should a patient require permanent discontinuation of either agent, the patient will come off protocol treatment.

6.1. Dose modification for non-hematological toxicity

- Any Grade ≥ 4 non-hematological toxicity
- Any Grade 3 non-hematological toxicity with the exception of
  - Grade 3 nausea, vomiting, and or diarrhea that is controlled within 72 hours
  - Grade 3 elevations in ALT/AST that returns to meet initial eligibility criteria within 7 days of study drug interruption and do not recur upon study re-challenge.
  - Grade 3 Fever or infection < 5 days duration
  - Grade 3 electrolyte imbalances that respond to oral or intravenous supplementation
  - Asymptomatic grade 3 creatinine kinase elevation, which remains <10 xULN.
- Grade 2 non-hematological toxicity that persists for >7 days and considered intolerable to the patient and/or not controlled with standard supportive measurements.
- Any non-hematological toxicity requiring treatment interruption for >21 days. The exception would be grade 3 weight loss as it is unlikely that the patient would return to baseline within 21 days. Patients who experience Grade 3 weight loss may resume study medication with a dose reduction if the patient is deriving benefit from therapy, the weight loss has stabilized and treating physician feels it is in the best interest of the patient.

If a patient experiences a non-hematological toxicity requiring dose modification, the attributable treatment(s) will be held. If the toxicity resolves to meet study parameters within 21 days after discontinuation, the patient may resume treatment at reduced dose. (See Table in 6.3 below). Doses reduced for toxicity will not be re-escalated even if there is minimal or no toxicity with the reduced dose. Held doses will not be made up for and the cycle remains 28 days.

If toxicity does not meet parameters to restart within 21 days, then patient is removed from protocol therapy.

Two dose reductions will be allowed for toxicity. If a patient experiences toxicity requiring modification after two dose reductions, the patient must discontinue protocol therapy.

6.2. Dose modification for hematological toxicity

- Grade 4 thrombocytopenia
- Any hematological toxicity requiring treatment interruption for >21 days.

If a patient experiences a hematologic toxicity requiring dose modification, the attributable treatment(s) will be held. CBC should be checked twice weekly during this time. When toxicity resolves to meet study parameters within 21 days of discontinuation, the patient may resume treatment at the reduced doses (See Table in 6.3 below).

Two dose reductions will be allowed for toxicity. If a patient experiences hematological toxicity requiring dose modification after two dose reductions, the patient must discontinue protocol therapy.

6.3. Dose reduction table

Dose Reduction Table			
AGENT	Current Dose	First dose reduction (% dose reduction)	Second dose reduction (% dose reduction)
Selumetinib BID continuous	50 mg	35 mg (30%)	25 mg (30%)
Sirolimus QD continuous	4 mg	3 mg (25%)	2 mg (33%)

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

7.1. Adverse Event and Laboratory Abnormalities

7.1.1. Clinical AE's

7.1.1.1. Definition of Adverse Events

Per the International Conference of Harmonisation (ICH), an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. Pre-existing conditions which worsen during a study are to be reported as AEs.

7.1.1.2. CTCAE term (AE description)

The descriptions found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

7.1.1.3. Intensity

Intensity of all adverse events will be graded according to the NCI CTCAEv 4.0 on a five-point scale (grades 1 to 5) and reported in detail on the CRF. Adverse events not listed on the CTCAE should be graded as follows:

CTC Grade	Equivalent To:	Definition
Grade 1	Mild	Discomfort noticed but no disruption of normal daily activity
Grade 2	Moderate	Discomfort sufficient to reduce or affect daily activity; no treatment or medical intervention is indicated although this could improve the overall well-being or symptoms of the patient
Grade 3	Severe	Inability to work or perform normal daily activity; treatment or medical intervention is indicated in order to improve the overall well-being or symptoms; delaying the onset of treatment is not putting the survival of the patient at direct risk.
Grade 4	Life threatening/ disabling	An immediate threat to life or leading to a permanent mental or physical conditions that prevents work or performing normal daily activities; treatment or medical intervention is required in order to maintain survival.
Grade 5	Death	AE resulting in death

7.1.1.4. Drug-Adverse Event relationship

The causality relationship of study drug to the adverse event will be assessed by the investigator as either:

Yes or No

If there is a reasonable suspected causal relationship to the study medication, i.e. there are facts (evidence) or arguments to suggest a causal relationship, drug-event relationship should be assessed as **Yes**.

The following criteria should be considered in order to assess the relationship as **Yes**:

– Reasonable temporal association with drug administration

– Known response pattern to suspected drug

– Disappears or decreases on cessation or reduction in dose

– Reappears on rechallenge

The following criteria should be considered in order to assess the relationship as **No:**

– It does not follow a reasonable temporal sequence from administration of the drug.

– It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.

– It does not follow a known pattern of response to the suspected drug.

It does not reappear or worsen when the drug is readministered.

If an investigator's opinion there is no relationship to selumetinib or sirolimus therapy, an alternate etiology must be provided for the adverse event.

7.1.1.5. Definition of Serious Adverse Events

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any Adverse Event that at any dose fulfills at least one of the following criteria:

- is fatal; (results in death; NOTE: death is an outcome, not an event)

- is Life-Threatening (NOTE: the term "Life-Threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe).

- required in-patient hospitalization or prolongation of existing hospitalization;

- results in persistent or significant disability/incapacity;

- is a congenital anomaly/birth defect;

- is medically significant or requires intervention to prevent one or other of the outcomes listed above.

Unexpected adverse events are any adverse events, which have not been reported as being associated selumetinib or sirolimus.

7.1.1.6. Progression of Underlying Malignancy

Progression of underlying malignancy is not reported as an adverse event if it is clearly consistent with the suspected progression of the underlying cancer as defined by RECIST criteria, or other criteria as determined by protocol. **Hospitalization due solely to the progression of underlying malignancy should NOT be reported as a serious adverse event.** Clinical symptoms of progression may be reported as adverse events if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.

Symptomatic deterioration may occur in some patients. In this situation, progression is evident in the patient's clinical symptoms, but is not supported by the tumor measurements. Or, the disease progression is so evident that the investigator may elect not to perform further disease assessments. In such cases, the determination of clinical progression is based on symptomatic deterioration. These determinations should be a rare exception as every effort should be made to document the objective progression of underlying malignancy.

If there is any uncertainty about an adverse event being due only to the disease under study, it should be reported as an AE or SAE.

7.1.1.7. Treatment and Follow-up AEs

After the discontinuation of therapy continue to follow up AEs as follows:

Related AEs: Follow until one of the following occurs:

- Resolved or improved to baseline

- Relationship is reassessed as unrelated

- Death

- Start of new anti-cancer regimen

- Investigator confirms that no further improvement can be expected

- Clinical or safety data will no longer be collected, or final database closure

Unrelated severe or life threatening AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Severity improved to grade 2
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated Grade 1 or Grade 2 AEs: Follow as clinically indicated.

The final outcome of each adverse event must be recorded on the CRF

7.1.1.8. Laboratory Test Abnormalities

Laboratory test results will be recorded on the laboratory results pages of the CRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event (SAE) should be reported as such.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded on the adverse event page in the CRF:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment)

7.1.1.9. Follow-up of Abnormal Laboratory Test

In the event of medically significant unexplained abnormal laboratory test values, the test should be repeated and followed until it has returned to the normal range, baseline value and/or an adequate explanation of the abnormality is found. If a clear explanation is established it should be recorded in the CRF.

7.2. Handling of Safety Parameters

7.2.1. Reporting of Adverse Events

All adverse events (related and unrelated) ≥ Grade 3 occurring during the study and up to 30 days after the last dose of study medication must be reported. Reporting the specific time of onset of a given AE is only necessary when it occurs in relation to study drug administration.

7.2.2. Reporting of Serious Adverse Events (immediately reportable)

Any clinical adverse event or abnormal laboratory test value that is serious and which occurs during the course of the study (as defined in section 7.1.1.5 above), must be reported to the Principal Investigator(s), SARC and AstraZeneca within one working day of the investigator becoming aware of the event (expedited reporting). If only limited information is initially available, follow-up reports are required. The original SAE Form must be kept on file at the study site.

SAE's must be reported on the MedWatch Form 3500A along with the completed Fax Coversheet and faxed to all parties listed above. (See Operations Manual)

Related Serious Adverse Events MUST be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed.

Unrelated Serious Adverse Events must be collected and reported during the study and for up to 30 days after the last dose of study medication.

7.2.3. Reporting of all unanticipated problems involving risk to subjects or others (UPIRTSOs) to the HRPO

The HRPO Reporting requirements ask only for UPIRTSOs to be reported: "All unanticipated problems involving risk to subjects or others must be promptly reported by telephone (301-619-2165), by email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), or by facsimile (301-619-7803) to the HRPO. A complete written report will follow the initial notification. In addition to the methods above, the complete report can be sent to the US Army Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000."

7.2.4. Pregnancy

Females must be instructed to stop taking the study medication and immediately inform the investigator if pregnancy occurs during the study. Pregnancies occurring up to 120 days after the completion of the study medication must also be reported to the investigator. The investigator should report all pregnancies within 24 hours to the sponsor.

The investigator should counsel the patient; discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy.

Pregnancy occurring in the partner of a male patient participating in the study should also be reported to the investigator and the sponsor. The partner should be counseled and followed as described above.

7.3. Warnings and Precautions

7.3.1. Selumetinib (AZD6244)

Selumetinib (AZD6244 Free base [NSC 741078], AZD6244 Hydrogen sulfate [NSC 748727])

Risks and side effects related to selumetinib taken from IB. The risks of treatment with selumetinib are from pooled data from completed phase I and II studies. In parentheses are % of all CTC grade adverse events (N=355).

Skin and cutaneous events: all rashes (72.4), acneiform rash (54.4), dry skin (13), paronychia (2.5)

Gastrointestinal: diarrhea (50.4), nausea (47), vomiting (31), stomatitis (9), dry mouth (8.2).

General: asthenic events (43.1), peripheral edema (33.5), facial edema (13), pyrexia (11.5).

Respiratory: dyspnea (20)

Eye: blurred vision (6.2). Generally grade 1. AEs consistent with retinal vein occlusion have been reported in a small number of patients receiving selumetinib, generally in combination with other anticancer agents targeting AKT or IGFR-1R inhibition or with cytotoxic chemotherapy.

Investigations: hyperphosphatemia (0.3), increased blood pressure (10.1), AST increased (4.5), ALT increased (4.8), hypoalbuminemia (1.1), reduced LVEF (2.0), CK increased (55.1)

7.3.2. Sirolimus

Side effects appear to be related to drug concentration and improves with maintenance of levels between 10 to 20 ng/mL. Sirolimus' effect on the developing fetus is not known and is not recommended for administration to nursing mothers. Patients should not be administered live vaccines.

Table 1. Most Common (≥ 30%) Adverse Reactions Observed

Side Effect Term*	Percentage out of 100 Patients
Peripheral edema	58
Hypertriglyceridemia	57

Hypertension	49
Hypercholesterolemia	46
Creatinine increased	40
Constipation	38
Abdominal pain	36
Diarrhea	35
Fever	34
Headache	34
Anemia	33
Pain	33
Urinary tract infection	33
Arthralgia	31
Nausea	31
Thrombocytopenia	30

Table 2. Less Likely (≥ 3%, but < 20%) Adverse Reactions Observed

Side Effect Term*
Azoospermia
Capillary leak syndrome
Epistaxis
Febrile neutropenia
Fluid accumulations
Hyponatremia/hypokalemia
Hypophosphatemia
Hypotension
Immune suppression and resulting risk for opportunistic infections
Leukopenia
Pneumonitis
Stomatitis
Thrombotic thrombocytopenic
Vomiting
Wound dehiscence

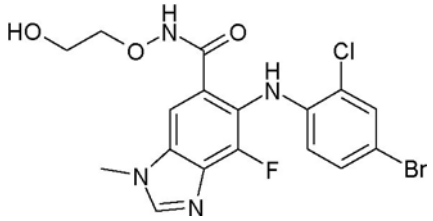
*From rxlist.com

8. PHARMACEUTICAL INFORMATION

8.1. Selumetinib (AZD6244)

CHEMICAL NAME: 6-(4-Bromo-2-chloro-phenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxy-ethoxy)-amide
OTHER NAMES: AZD6244, ARRY-142886, AR00142866, AR-142886-01

CHEMICAL STRUCTURE:



MECHANISM OF ACTION: The RAS/RAF/MEK/ERK pathway is an important mediator of many cellular processes including proliferation, survival, differentiation, apoptosis, motility, and metabolism. This pathway is often aberrantly activated in human tumors due to the overexpression of activated K-RAS, mutant b-Raf, or other growth factor receptors. AZD6244 hyd sulfate is a selective mitogen-activated protein kinase kinase (MEK) inhibitor. By inhibiting MEK, AZD6244 hyd sulfate inhibits ERK phosphorylation. Thus, AZD6244 hyd sulfate may inhibit oncogenic growth signaling in tumor cells by targeting the RAS/RAF/MEK/ERK pathway.

MOLECULAR FORMULA: C17H15BrClFN4O3

MOLECULAR WEIGHT: 457.7

8.1.1. Preparation and Administration

Selumetinib is administered orally twice daily. A cycle is defined as 28 consecutive days starting with the first day of treatment (day 1). The dose of selumetinib is fixed based on dose level. Treatment will be administered in an outpatient setting. Selumetinib should be taken with water (Approximately 4 to 8 ounces).

ROUTE OF ADMINISTRATION: Oral. Take selumetinib on an empty stomach (either 1 hour before or 2 hours after meals). selumetinib capsules should be taken with water only.

POTENTIAL DRUG INTERACTIONS: High vitamin E doses may potentiate warfarin's anticoagulant activity. Monitor PT/INR more frequently in patients receiving both warfarin and selumetinib capsules.

Avoid concomitant intake of Vitamin E in excess of 100% of the recommended daily dose.

8.1.2. Formulation, Packaging and Labelling

HOW SUPPLIED: The drug product consists of series of plain, hydroxypoplymethylcellulose (HPMC) 60 count capsules containing 10 mg (white) and 25 mg (blue) Selumetinib in each bottle (expressed as free base) for oral administration. Selumetinib capsules are supplied in white, high density polyethylene (HDPE) bottles with desicant, tamper evident induction seal membrane and child resistant screw closure.

STORAGE: Capsules should be stored in their original packaging until use. For further information, please refer to the investigational product label.

STABILITY: Stability studies are ongoing.

AstraZeneca will send unlabeled bottles and the sponsor must arrange their own labeling.

8.2. Sirolimus

LABORATORY CODE:. The chemical name of sirolimus (also known as rapamycin) is (3S,6R ,7E,9R ,10R,12R ,14S,15E ,17E,19E ,21S,23S ,26R,27R ,34aS)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34, 34a-hexadecahydro-9,27-dihydroxy-3-[(1R)-2-[(1S,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3H-pyrido[2,1-c][1,4] oxaazacyclohentriacontine-1,5,11,28,29 (4H,6H ,31H)-pentone. Its molecular formula is C51H79NO13 and its molecular weight is 914.2

MECHANISM OF ACTION: Sirolimus is a macrocyclic lactone produced by Streptomyces hygroscopicus and inhibitor of mammalian Target of Rapamycin (mTOR) serine threonine kinase, which plays a critical role in regulating cellular energy sensing, growth and metabolism.

CLASSIFICATION: mTORC1 inhibitor

8.2.1. Preparation and Administration

For this trial: 2 and 1 mg tablets may be used.

Food effects: In 22 healthy volunteers receiving Rapamune Oral Solution, a high-fat meal (861.8 kcal, 54.9% kcal from fat) altered the bioavailability characteristics of sirolimus. Compared with fasting, a 34% decrease in the peak blood sirolimus concentration (Cmax), a 3.5-fold increase in the time-to-peak concentration (tmax), and a 35% increase in total exposure (AUC) was observed. After administration of Rapamune Tablets and a high-fat meal in 24 healthy volunteers, Cmax, tmax, and AUC showed increases of 65%, 32%, and 23%, respectively. To minimize variability, tablets should be taken consistently with or without food.

8.2.2. Formulation, Packaging and Labelling

Tablets should be stored at 20° to 25°C (USP Controlled Room Temperature) (68° to 77°F). Use cartons to protect blister cards and strips from light. Dispense in a tight, light-resistant container as defined in the USP.

8.3. Agent Ordering

For this trial, selumetinib will be provided by AstraZeneca. SARC through a third party vendor will ship drug directly to participating sites. Details will be provided in the operations manual.

Sirolimus is commercially available. For this study, sirolimus will be supplied through the study (SARC to ship through third party vendor) or can be purchased commercially through your pharmacy. For agent ordering, see operations manual.

8.4. Agent Accountability

Accountability and patient compliance will be assessed by maintaining adequate drug dispensing and return records. Compliance with individual patient dosing is assured as the drug is administered intravenously and recorded at the clinical site.

Accurate records must be kept for each study drug provided by the sponsor. The drug dispensing log must be kept current and contain the following information:

- documentation of drug shipments received from the sponsor (date received and quantity)
- disposition of unused study drug not dispensed to patient
- the identification of the patient to whom the study medication was dispensed
- the date(s) and quantity of the study medication dispensed to the patient

This inventory must be available for inspection by the Monitor. All supplies, including partially used or empty containers and copies of the dispensing & inventory logs, must be returned to the SARC Monitor at the end of the study, unless alternate destruction has been authorized by SARC, or required by local or institutional regulations

8.5. Destruction study drugs

Local or institutional regulations may require immediate destruction of used investigational product for safety reasons e.g., cytotoxicity. In these cases, it may be acceptable for investigational site staff to destroy dispensed investigational product before a monitoring inspection provided that source document verification is performed on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, returned and destroyed. Written authorization must be obtained from the sponsor at study start up before destruction.

Written documentation of destruction must contain the following:

- Identity (batch numbers or patient numbers) of investigational product(s) destroyed
- Quantity of investigational product(s) destroyed
- Date of destruction (date discarded in designated hazardous container for destruction)
- Method of destruction (the site must provide the sponsor with documentation of their institutional policy and procedures for handling and disposing of hazardous drugs)
- Name and signature of responsible person (or company) who destroyed investigational products(s)

A list of the adverse events and potential risks associated with Study Agent can be found in Section 7.1.

9. CORRRELATIVE / SPECIAL STUDIES

9.1. Laboratory Correlative Studies

Some of the following correlative studies are mandatory for trial participation, and some are optional and will only be performed in patients providing informed consent.

Details regarding collection and assessment will be provided in the operations manual.

After analyses, any remaining correlative samples may be retained in a SARC designated specimen bank with the consent of the patient. No personal health information will be linked to the sample. The specimen will be marked with the patient study identification number only.

9.1.1. Mandatory

9.1.1.1. Imaging FDG-PET will be obtained at baseline and on Cycle 1 Day 11 (±3) to assess for early FDG-PET response. If baseline FDG-PET is negative (avidity is not measureable), then second FDG-PET is not required.

Imaging: For patients who have MRI imaging done for tumor disease evaluation, DWI/ADC mapping sequence will be performed if feasible (mandatory if feasible) at baseline and at the time of disease evaluation. This sequence will add less than 3 minutes of scanning time and will explore the value of functional MRI imaging technology.

9.1.1.2. Patient reported pain and impact measurements

The patient reported pain evaluation will consist of two validated scales. The numerical rating scale-11 (NRS-11) will be used to assess pain severity, and the PROMIS pain interference scale (PROMIS-PI) will be used to assess the impact of pain on daily activities. Total administration time is approximately 5 minutes. These tests will be given prior to treatment and then prior to cycles 2, 3, 5, 9, 13, and then every 6 cycles. On this study, the NRS-11 will

be administered to patients of all ages, the pediatric PROMIS-PI will be administered to children ages 12-17 years and the adult PROMIS-PI will be administered to adults 18 years and older.

9.1.1.3. Blood samples for ctDNA will be collected at baseline and then with disease evaluation (prior to cycles 3, 5, 7, and then every 3 cycles).

9.1.1.4. Blood samples will be collected at baseline and on Cycle 1 Day 15 (once between Cycle 1 Day 15 thru 28) to assess the following:

- PBMCs will be assessed using flow cytometry for immune subsets.
- Circulating endothelial progenitor cells (CEP) and mature circulating endothelial cells (CEC) will be assessed by flow cytometry.
- Circulating Tumor Cells (CTCs) will be assessed using ferrofluidic enrichment and flow cytometry.
- Peripheral blood will be analyzed using the NanoString 770 gene Cancer Immune Panel to evaluate immune gene signature

9.1.2. Optional

Tumor sample will be collected at baseline and on Cycle 1 Day 15 (or once between Cycle 1 Day 15 thru 28) for consenting patients with tumor safely accessible by percutaneous biopsy.

A minimum of two biopsy cores are required for analysis at each time point and will be prioritized depending on number of biopsy cores obtained.

Tissue Correlatives (listed in order of priority based on tissue availability)

- Analysis of immune gene signature using the NanoString nCounter® platform to analyze immune gene signature.
- Evaluation of protein levels and MEK target inhibition.
- Genomic and epigenetic profiling

10. STUDY EVALUATIONS AND STUDY CALENDAR

10.1. SCREENING STUDIES

The following procedures will be performed within **4 weeks** prior to start of enrollment unless otherwise stated:

- Informed consent
- Review concomitant medications
- FDG-PET
- Baseline radiologic imaging: Evaluation of other measurable or evaluable disease sites by appropriate radiological evaluation (i.e. CT scan, MRI)

The following procedures will be performed within **14 days** prior to start of enrollment unless otherwise stated:

- Serum Pharmacodynamic and Immune Markers: Mandatory blood samples for pharmacodynamics, genomics and immune infiltrates
- Tumor biopsy: Optional serum/tissue samples for pharmacodynamics, genomics and immune infiltrates
- History/demographics
- Physical exam: vital sign, height, weight, body surface area
- Performance status: Lansky/Karnofsky (Appendix I)
- Hematology: complete blood count (CBC), differential
- Serum Chemistry: ALT, AST, alkaline phosphatase, bilirubin, BUN, creatinine, electrolytes, calcium, magnesium, phosphorus, total protein, albumin, serum creatinine phosphokinase.
- Fasting* glucose and Fasting serum lipid profile (triglycerides, total cholesterol, HDL and LDL) *Fasting = 12 hours prior to glucose testing and lipid testing
- Echocardiogram or Cardiac MRI and ECG (performed within 4 weeks prior to start of enrollment)
- Ophthalmology evaluation: A complete ophthalmology evaluation will be performed pre-treatment. Particular emphasis will be given to the evaluation of corneal opacifications and retinal changes.
- Patient reported pain severity and pain interference assessments.

The following procedures will be performed within **7 days** prior to start of therapy unless otherwise stated:

- Serum or urine pregnancy: required for females of childbearing potential.

NOTE: Eligibility labs/evaluations performed can be counted as pre-treatment evaluations as performed in time frame requested.

10.2. ON STUDY EVALUATIONS

The following procedures will be performed during the treatment period:

Weekly During Cycle 1:

- History
- Physical exam: vital signs
- Hematology: complete blood count, differential. If patient has Grade 4 neutropenia or thrombocytopenia, then CBC should be checked every 3-4 days until recovery to Grade 3.

- Serum Chemistry: ALT, AST, alkaline phosphatase, bilirubin, BUN, creatinine, electrolytes, calcium, magnesium, phosphorus, total protein, albumin, serum creatinine phosphokinase.
- Patient diary
- Assessing of AEs

Cycle 1 Day 11 ± 3 days
- FDG-PET

Cycle 1 Day 15 (up to day 28)
- Serum Pharmacodynamic and Immune Markers: Mandatory blood samples for pharmacodynamics, genomics and immune infiltrates
- Tumor Biopsy: Optional serum/tissue samples for pharmacodynamics, genomics and immune infiltrates

Prior to Subsequent Cycles*
- History
- Physical exam: vital signs, height, weight, body surface area
- Performance status: Lansky/Karnofsky (Appendix I)
- Hematology: complete blood count, differential. If patient has Grade 4 neutropenia or thrombocytopenia, then CBC should be checked every 3-4 days until recovery to Grade 3.
- Serum Chemistry: ALT, AST, alkaline phosphatase, bilirubin, BUN, creatinine, electrolytes, calcium, magnesium, phosphorus, total protein, albumin, serum creatinine phosphokinase.
- Fasting glucose and Fasting serum lipid profile (triglycerides, total cholesterol, HDL and LDL). Fasting = 12 hours prior to glucose testing and lipid testing
- Serum or urine pregnancy: required for females of childbearing potential.
- Patient diary
- Assessing of AEs

Prior to Cycles 3, 5, 7 and then every 3 cycles (within 7 days prior to start of subsequent cycle)
- ctDNA blood sample
- Radiologic imaging: Evaluation of other measurable or evaluable disease sites by appropriate radiological evaluation (i.e. CT scan, MRI)

Prior to Cycles 2, 3, 5, 9, 13, and then every 6 cycles
- Patient reported pain severity and pain interference assessments.

Prior to Cycles 6 and 12, and then every 6 cycles (within 14 days prior to start of subsequent cycle)
- Echocardiogram or Cardiac MRI and ECG
- Ophthalmology evaluation: Particular emphasis will be given to the evaluation of corneal opacifications and retinal changes.

*All evaluations can be obtained within 72 hours prior to start of a subsequent cycle, unless otherwise stated. All efforts should be made to adhere to the schedules listed. However, to provide flexibility to changes in patient's schedules and holidays for long term treatment, +/- 2 day changes will not be considered a protocol deviation and will not require reporting to IRB or FDA.

End of Therapy

- History
- Physical exam: vital signs, height, weight, body surface area
- Performance status: Lansky/Karnofsky (Appendix I)
- Hematology: complete blood count, differential
- Serum Chemistry: ALT, AST, alkaline phosphatase, bilirubin, BUN, creatinine, electrolytes, calcium, magnesium, phosphorus, total protein, albumin, serum creatinine phosphokinase.
- Fasting* glucose and Fasting serum lipid profile (triglycerides, total cholesterol, HDL and LDL) *Fasting = 12 hours prior to glucose testing and lipid testing
- Patient diary

10.3. OFF STUDY EVALUATIONS

The following studies should be performed, if feasible, at the time a patient is removed from the study:

- History
- Physical exam: vital signs, height, weight, body surface area
- Performance status: Lansky/Karnofsky (Appendix I)
- Hematology: complete blood count, differential
- Serum Chemistry: ALT, AST, alkaline phosphatase, bilirubin, BUN, creatinine, electrolytes, calcium, magnesium, phosphorus, total protein, albumin, serum creatinine phosphokinase.
- Radiologic imaging: Evaluation of other measurable or evaluable disease sites by appropriate radiological evaluation (i.e. CT scan, MRI)

STUDY CALENDAR

Studies to be obtained	Pre-treatment[1]	During Cycle 1	Prior to subsequent cycles	End of therapy
History and Physical with vitals	X	Weekly	X	X
Performance status (Karnofsky)	X		X	X
CBC, differential	X	Weekly[2]	X[2]	X[2]
Serum Chemistry[3]	X	Weekly	X	X
Fasting glucose and serum lipid profile[4]	X		X	X
Urine or serum pregnancy[5] test	X		X	
Echocardiogram or cardiac MRI and ECG	X[6]		Prior to cycles 6 and 12, and every 6 cycles	
FDG-PET	X[6]	Day 11 +/- 3 days		
Ophthalmology Exam	X		Prior to cycles 6 and 12 and every 6 cycles	
Tumor Evaluation (by CT, MRI)	X[6]		Prior to cycles 3,5,7 and then every 3 cycles	
Patient reported pain	X		Prior to cycles 2, 3, 5, 9, 13, and then every 6 cycles	
Patient Diary		Weekly	X	X
Pharmacodynamic/Immune Markers[7]	X	Day 15 (up to day 28)[10]	ctDNA only: prior to cycles 3, 5, 7 and then every 3 cycles	
Tumor biopsy[8]	X	Day 15 (up to day 28) [10]		
Genomic testing[9]	X	Day 15 (up to day 28)[10]		

[1] See Section 3.2

[2]For patients who experience Grade 4 ANC or platelets, check CBC q 3-4 days until ≥ Grade 3

[3]Sodium, potassium, chloride, bicarbonate, calcium, phosphorus, magnesium, creatinine, glucose, blood urea nitrogen, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, uric acid.

[4]Fasting = 12 hours prior to testing. Glucose and serum lipid panel (triglycerides, total cholesterol, HDL, LDL)

[5] For females of child-bearing potential

[6]Can be done within 4 weeks prior to enrollment.

[7] For serum and/or tissue samples, See Section 2.5

[8] Tumor biopsy is optional and will be collected only for patients who provide written consent and has tumor accessible to percutaneous biopsy

[9] Baseline blood will be (mandatory) collected and for patients who provide written consent and has tumor accessible to percutaneous biopsy may be collected at baseline and day 15 per patient consent

[10] Will be collected during cycle 1 on any one day on or after day 15 (i.e. one day between day 15-28)

11. MEASUREMENT OF EFFECT

11.1. Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response prior to every odd cycle.

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) [65]. 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 RECIST criteria.

11.1.1. Definitions

Evaluable for PET response: Patient must have FDG-PET performed at time points stated in protocol to be evaluable for PET response. PET response will be measured by PERCIST 1.0 (see section 11.1.6).

Evaluable for objective response: Any patient who is enrolled and receives at least one dose of selumetinib and one dose sirolimus will be considered evaluable for response provided: (1) the patient demonstrates progressive disease or death while on protocol therapy; or (2) the patient is observed on protocol therapy for at least one cycle and the tumor is not removed surgically prior to the time complete response or partial response is confirmed; or (3) the patient demonstrates a complete or partial response or stable disease as confirmed according to protocol criteria. The evaluation period for determination of best response will be 6 treatment cycles. All other patients will be considered non-responders.

Evaluable for toxicity: All patients who receive at least one dose of selumetinib or sirolimus and either experience a toxicity or complete the first cycle with no toxicity will be considered in the evaluation of toxicity.

11.1.2. Disease Parameters

Measurable Disease:
The presence of at least one lesion that can be accurately measured in at least one dimension with the longest diameter at least 10mm (CT scan slice thickness no greater than 5mm). The investigator will identify up to 5 measureable lesions to be followed for response (maximum 2 per organ). Previously irradiated lesions must demonstrate clear evidence of progression to be considered measureable.

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

Non-measureable disease:

All other lesions (or sites of disease) including small lesions (<10mm or pathological LN ≥10 to <15mm short axis) are considered non-measureable. Bone lesions, leptomeningeal lesions, ascites, pleural/pericardial effusion, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI) are considered non-measureable.

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 measureable lesions up to a maximum of 2 lesions per organ and 5 lesions 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 lead 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 that can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum of 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 measureable dimension of the disease.

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

11.1.3. Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation. The same method of assessment and same technique should be used to characterize each identified and reported lesion at baseline and during follow up.

11.1.4. Response Criteria

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

Partial Response (PR): A > 30% decrease in the sum of the diameters of target lesion, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the target lesions, taking reference the smallest sum on study (this includes baseline if that is the smallest on study). In addition to the relative increase in 20%, the sum must also demonstrate an absolute increase of at least 5mm. The appearance of one or more new lesions is also considered progression. In the presence of SD or PR in the target disease, but unequivocal progression in non-target or non-measureable disease, the patient has PD if there is an overall level of substantial worsening in non-target disease such that the overall tumor burden has increased sufficiently to merit discontinuation of therapy.

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.

Evaluation of Best Overall Response

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Response for this Category also requires:
CR	CR	No	CR	≥ 4 weeks confirmation
CR	Non-CR/ Non-PD	No	PR	
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	
* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.				

11.1.5. Duration of Response

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

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent 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.

11.1.6. PERCIST 1.0 Response [46]

Measurability of lesions at baseline: 1) Measurable target lesion is hottest single tumor lesion SUL of "maximal 1.2 cm diameter volume ROI in tumor" (SUL peak). SUL peak is at least 1.5 fold greater than liver SUL mean +2 SDs (in 3 cm spherical ROI in normal right lobe of liver). If liver is abnormal, primary tumor should have uptake > 2.0 x SUL mean of blood pool in 1 cm diameter ROI in descending thoracic aorta extended over 2 cm z axis. 2) Tumor with maximal SUL peak is assessed after treatment. Although typically this is in the same region of tumor as that with highest SUL peak at baseline, it need not be. 3) Uptake measurements should be made for peak and maximal single-voxel tumor SUL. Other SUV metrics, including SUL mean at 50% or 70% of SUV peak can be collected as exploratory data; TLG can be collected ideally on basis of voxels more intense than 2 SDs above liver mean SUL (see below). 4) These parameters can be recorded as exploratory data on up to 5 measureable target lesions, typically the 5 hottest lesions.

Normalization of uptake: Normal liver SUL must be within 20% (and <0.3 SUL mean units) for baseline and follow up study to be assessable. If liver is abnormal, blood-pool SUL must be within 20% (and <0.3 SUL mean units) for baseline and follow up study to be assessable. Uptake time of baseline and follow up study must be within 15 minutes of each other to be assessable. Same scanner or model at same site, injected dose, acquisition protocol and software should be used.

Complete Metabolic Response (CMR): Complete resolution of FDG update within measureable lesion so that it is less than mean liver activity and indistinguishable from surrounding background blood pool levels. Disappearance of all other lesions to background blood pool levels. No new FDG avid lesions.

Partial Metabolic Response (PMR): Reduction of minimum of 30% in target measureable tumor FDG SUL peak. Absolute drop must be at least 0.8 SUL units as well. Measurement is commonly in same lesion at baseline but can be another

lesion if that lesion was previously present and is the most active lesion after treatment. No new lesions.

Progressive Metabolic Disease (PMD): >30% increase in FDG SUL peak, with >0.8 SUL unit increase in tumor SUV peak from baseline scan in pattern typical of tumor and no of infection/treatment effect.

Stable Metabolic Disease (SMD): Not CMR, PMR or PDDATA REPORTING / REGULATORY CONSIDERATIONS

12. DATA REPORTING / REGULATORY CONSIDERATIONS

12.1. Method

A final study report describing the outcome of the trial will be created at the end of the study.

12.2. Data, Safety and Monitoring

SARC is responsible for overseeing the conduct of this trial which includes review of data, safety and monitoring for this trial. SARC Clinical Trials Review Committee convenes monthly and will provide safety oversight for this trial. The purpose of the Clinical Trials Review Committee is to review the status of the on-going SARC studies, which includes, but is not limited to:

- Review of all safety data (Serious Adverse Events reported)
- Review of protocol deviations/violations
- Review of study progress/accrual
- Discussion of statistical aspects of all protocols

The committee is chaired by the SARC Medical Officer, who is responsible for leading the meeting and providing medical oversight. Attendance includes all Principal Investigators on active SARC studies, SARC Research Project Managers, SARC President, and a biostatistician.

Safety oversight for this trial is also supported by the SARC Clinical Research Committee which is made up of senior sarcoma investigators and the SARC President and Chief Executive Officer. This committee is provided with the clinical trial review committee minutes monthly for their review, and also convenes quarterly. The medical officer updates the committee on the ongoing clinical trial status as well as any areas of concern particularly related to safety. This committee provides an additional level of medical oversight for this trial.

The SARC Medical Officer will also be the Medical monitor for this study.

The medical monitor is required to review all unanticipated problems involving risk to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the Medical monitor must comment on the outcomes of the event or

problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The Medical monitor must also indicate whether he/she concurs with the details of the report provided by the principal investigator. Reports for events determined by either the investigator or medical monitor to be related to participation and reports of events resulting in death must be promptly forwarded to the sponsor. SARC will be responsible for forwarding reports to the SARC medical officer.

The SARC Medical Officer will also function as the Department of Defense (DoD) required "Independent Research Monitor". The DoD Independent Research Monitor will be responsible evaluating any risks or concerns of the research in addition to overseeing the safety of the research and reporting observations/findings to the IRB of Record or a designated official. The DoD Independent Research Monitor will review all unanticipated problems involving risk to volunteers or others associated with the protocol and provide an unbiased written report of the event to the IRB of Record. The DoD Independent Research Monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research. The DoD Independent Research Monitor shall have authority to stop the research protocol in progress, remove individual human subjects from the study, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report. The DoD Independent Research Monitor is responsible for promptly reporting their observations and findings to the IRB.

DoD Independent Research Monitor functions may include:

- Observing recruitment and enrollment procedures and the consent process for individuals, groups or units,
- Overseeing study interventions and interactions,
- Reviewing monitoring plans and UPIRTSO reports;
- Overseeing data matching, data collection, and analysis

At a minimum, the DoD Independent Research Monitor:

- May discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research;
- Shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report;
- Shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO.

Sites with patients on study will be monitored at least once during the duration of the study. Selected patient charts as well as the participating institution's Standard Operating Procedures may be monitored at the time of the visit. Data from participating institutions should be available when the protocol is monitored.

The institutional principal investigator is responsible for having all records and data for all patients enrolled at his/her institution available at that institution for monitoring.

Participating study sites will be informed of findings on a regular basis and be provided with ample information to report to their local IRB in accordance with local site policies.

The knowledge of any pending compliance inspection/visit by the FDA, OHRP, or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to USAMRMC ORP HRPO.

12.3. Patient Accrual and Participating Centers

The study would be conducted through the SARC consortium in collaboration of the NF Consortium with 10-15 consortium centers.

12.4. Multi-institutional Guidelines

The trial coordinating center (Operations Center) will be SARC. Patients will be registered electronically via the study website, and adverse events (as defined in Section 7) will be reported to the operations center.

IRB Approvals
SARC will be the Operations Center. The protocol must be approved at the treating institution prior to enrolling patients. Documentation of individual institutional IRB approval, for the current protocol must be provided to the SARC Operations Office prior to enrolling patients on the trial. They may be provided via e-mail, fax, or US Mail. In addition, documentation of approval of all protocol amendments and of yearly continuing review must be provided to the SARC Operations Office Research Project Manager to allow patient entry. The mailing address is:

SARC
24 Frank Lloyd Wright Drive, PO Box 406
Ann Arbor, MI 48105
Phone: 734-930-7600
Fax: 734-930-7557
Email: SARC031@sarctrials.org

USAMRMC HRPO Approvals
As this trial receives funding by the Department of Defense, approval of the protocol must be obtained from the USAMRMC ORP HRPO in addition to the institutional IRB prior to implementation. Documentation of individual institutional IRB approval, for the current protocol must be provided to SARC at the Operations Center prior to enrolling patients on the trial. In addition,

documentation of approval of all protocol amendments and of yearly continuing review must be provided to the Operations Center to allow patient entry. They may be submitted via e-mail, fax, or US Mail. SARC will submit these documents to the USAMRMC Office of Research Protections (ORP), Human Research Protections Office (HRPO).

Major modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the USAMRMC ORP HRPO for approval prior to implementation. All other amendments will be submitted with the continuing review report to the USAMRMC ORP HRPO for acceptance. A copy of the approved continuing review report and the local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available. A copy of the approved final study report and local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available.

Amendments and Consents
SARC will be the Operations Center. IRB approval of the current protocol, protocol amendments, and yearly continuing review must be provided to the SARC Operations Office. In addition, a copy of the currently approved informed consent of each participating site will be kept on file at SARC. SARC will submit these documents to the USAMRMC ORP HRPO.

Patient Registration
Patient Registration will be centrally managed by the Operations Center electronically via the study website.

Data Collection and Toxicity Reporting
Registration reports will be generated by the Operations Center to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies by the study coordinator. Any potential problems will be brought to the attention of the Principal Investigator for discussion and action.

Access to the password protected study database will be limited to individuals involved in the clinical trial: SARC, Study PI, participating site PIs, and research nurses and data managers responsible for this trial.
Shipment and receipt of specimens and imaging studies sent for correlative studies will be entered on the study website and can thus be tracked.
A monthly phone conference will be held on an as needed basis between the Principal Investigator, the Operations Center, associate investigators, and participating sites to address QA issues, accrual, observed toxicities, and compliance with submission of required studies.

13. STATISTICAL CONSIDERATIONS

13.1. Study Design/Endpoints

The primary endpoint will be clinical benefit rate, which will be defined as a CR, PR, or stable disease ≥ 4 cycles. An evaluable patient will be classified as a responder (success) for the primary endpoint if the patient achieves a partial response (PR), complete response (CR), or stable disease (SD) at ≥ 4 cycles. The target clinical benefit rate will be 30% (p1=0.30), and a clinical benefit rate ≤ 5% (p0=0.05) will be considered uninteresting. Using a Simon's optimal two-stage phase II design with an alpha =0.10 (probability of accepting a poor treatment), and beta=0.10 (probability of rejecting a good treatment=0.10; power= 90%). Stage 1 will require 7 patients, with no further accrual if 0 of 7 respond. Since it may take several months to determine if a patient has responded, accrual may be temporarily paused while waiting to determine if there is at least one patient with a response among the 7 evaluable patients enrolled on stage 1. If ≥ 1/7 patients respond, accrual will continue until 21 patients have been enrolled. If ≥ 3/21 patients respond, this combination will be considered of sufficient activity, while 1-2 of 21 with benefit would be insufficient. Under the null hypothesis (p0=0.05), the expected sample size would be 11.2 patients, and the probability of early termination would be 69.8%.

Progression-Free Survival (PFS) is defined as the duration of time from the start of treatment to the time of objective progression or death.

13.2. Sample Size/Accrual Rate

Stage 1 will require 7 patients, with no further accrual if 0 of 7 respond. If > 1/7 patients respond, accrual will continue until 21 patients have been enrolled. If > 3/21 patients respond, this combination will be considered of sufficient activity, while 1-2 of 21 with benefit would be insufficient.

Patients of both genders, from all racial and ethnic groups are eligible for this trial if they meet the criteria outlined in Section 3.1. To date, there is no clinical information that suggests differences in selumetinib or sirolimus metabolism, disposition or disease response among racial or ethnic groups or between the genders, indicating that results of the trial will be applicable to all groups. Efforts will be made to extend the accrual to a representative population.

13.3. Analysis of primary endpoints

The clinical benefit rate will be determined by identifying if patients have a PR, CR, or stable disease for 4 or more cycles. This total will be divided by the number of patients evaluable for response, and this fraction will be reported along with a 95% two-sided confidence interval.

13.4. Analysis of Secondary Endpoints

Summary statistics will be used to describe the study population and baseline characteristics.

Progression free survival will be determined using the Kaplan-Meier method, with PFS at important time points (6 or 12 months for example) reported along with 95% two sided confidence intervals.

Toxicities to be summarized descriptively and tabulations on the type, severity, and relationship to study treatment will be performed.

Changes in FDG-PET avidity, tumor biomarkers, pain severity and pain interference ratings, and immune markers will be exploratory and summarized using descriptive statistics and when feasible compared using Wilcoxon rank test or pairwise t-test. Correlations to imaging response, toxicity, and immune markers will be made if feasible.

The comprehensive genomic analyses including next generation sequencing and ctDNA analysis will be performed in the Oncogenomics laboratory (Dr. Shern/NCI). Bioinformatics analysis will be primarily performed at the NCI using well-established pipelines previously developed.

13.5. Adverse event stopping rule

If in the first 7 patients, 3 or more patients experience a grade 4 or higher non-hematologic adverse event, the study team will suspend accrual (if the trial is still accruing) and do a thorough analysis of the adverse events. If the grade 4 or higher events are felt to be at least possibly related to the study treatment, the study team, in consultation with SARC scientific leadership will make a recommendation to the sponsor to either alter the dose level of one or more of the drugs or to stop the trial due to toxicity. All participating IRBs will be notified of the adverse event and the recommendation.

At any point after 8 or more patients are enrolled, 1/3 or more of the patients experience a grade 4 or higher non-hematologic adverse event felt to be at least possibly related to the study treatment, the study team will suspend accrual to evaluate the adverse events. In consultation with the SARCscientific leadership, the study team will recommend that the trial continue without modification, alter the dose level of one or more of the drugs, or to stop the trial due to toxicity. The recommendation will be made to the sponsor and all participating IRBs will be notified of the adverse events and the recommendation.

Adverse events are reviewed on at least a monthly basis by the study team and the SARC Clinical Trial Review Committee and the trial will stop accrual if the adverse event stopping boundary is crossed.

Adverse event stopping boundary summary:

- Stop when 3 patients experience grade 4 or higher non-hematologic adverse event if total number of patients accrued is 7 or less
- Stop when 1/3 of the patients experience grade 4 or higher non-hematologic adverse event if the total number of patients accrued is 8 or more

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APPENDIX I: PERFORMANCE STATUS CRITERIA

PERFORMANCE STATUS CRITERIA Karnofsky and Lansky performance scores are intended to be multiples of 10			
Karnofsky		**Lansky**	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

Patients >16 years old: use Karnofsky performance criteria

Patients ≤ 16 years old: use Lansky performance criteria

APPENDIX II: Restricted CYP and transporter co-medications

Cytochrome P450 and transporter inhibitor/inducer restrictions		
CYP/Transporter Category	**Drugs**	**Minimum drug wash-out period**
CYP3A4/5 Strong competitive inhibitors	Grapefruit juice, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, saquinovir, telithromycin and troleandomycin and voriconazole	1 week
	Idelalisib	2 weeks
CYP3A4/5 Strong time dependent inhibitors	Bocepravir, clarithromycin, cobicistat, danoprevir, elvitegravir, LCL161, lopinavir, mibefradil, posaconazole, ritonavir, telaprevir and tipranivir	2 weeks
CYP3A4/5 Strong inhibitors (classification unknown)	Conivaptan	1 week
CYP3A4/5 Moderate competitive inhibitors	Amprenavir, aprepitant, atazanavir, cimetidine, cyclosporine, fluconazole, imatinib and netupitant	1 week
CYP3A4/5 Moderate time dependent inhibitors	ACT-178882, casopitant, crizotinib, darunavir, diltiazem erythromycin, ledipasvir, lomitapide, tofisopam and verapamil	2 weeks
	FK1706	half-life not found
CYP3A4/5 Moderate inhibitors (classification not known)	ciprofloxacin and dronedarone	1 week
	Schisandra Sphenanthera	half-life not found
CYP3A4/5 Strong Inducers	Carbamazepine, phenytoin, rifabutin, rifampicin and St. John's Wort	3 weeks
	Enzalutamide and phenobarbital	5 weeks
	Mitotane	114 weeks
	Avasimibe	half-life not found
CYPA4/5 Moderate Inducers	Bosentan, genistein, lersivirine, lopinavir, modafinil, nafcillin, ritonavir, semagacestat, thioridazine and tipranavir	1 week

Cytochrome P450 and transporter inhibitor/inducer restrictions		
	Etravirine	2 weeks
	Efavirenez	3 weeks
	Talviraline	half-life not found
Pgp (MDR1) inhibitors	Dronedarone, erythromycin, indinavir, itraconazole, ketoconazole, lapatinib, lopinavir, ritonavir, quinidine and verapamil	1 week
	Vorapaxer	10 weeks
	Valspodar (PSC 833)	half-life not found
Pgp (MDR1) inducer	carbamazepine and rifampin	3 weeks
BRCP inhibitors	Atazanavir, cyclosporine, lopinavir, ritonavir, and tipranavir	1 week

Transporter Substrate Restrictions	
Transporters	**Substrates**
OATP (1B1 or 3)	Bosentan, fexofenadine, glyburide, pitavastatin, pravastatin,**repaglinide**, rosuvastatin
MATE(1 or 2K)	Cisplatin
Substrates in bold type have a narrow therapeutic index. Reference: Expert Opin Drug Metab Toxicol (2013) 9(6): 737-751. Washout periods should be 5x reported terminal half-lives.	