SARC Protocol #: **SARC023**

TITLE: A Phase I/II Trial of ganetespib in combination with the mTOR inhibitor sirolimus for patients with recurrent or refractory sarcomas including unresectable or metastatic malignant peripheral nerve sheath tumors

Sponsor: SARC (Sarcoma Alliance for Research through Collaboration)

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GLOSSARY OF ABBREVIATIONS

ALP	Alkaline phosphatase
ALT (SGPT)	Alanine aminotransferase
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate aminotransferase
AUC	Area under the curve
CBR	Clinical benefit rate
CI	Confidence interval
CR	Complete Response
DLT	Dose Limiting Toxicity
DoD	Department of Defense
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
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
MRI	Magnetic Resonance Imaging

GLOSSARY OF ABBREVIATIONS

MUGA	Multiple Gated Acquisition scan
NCIMUGA	National Cancer Institute Multiple Gated Acquisition scan
NCI-CTCNCI	National Cancer Institute-Common Toxicity Criteria National Cancer Institute
NCI-CTCAENCI-CTC	National Cancer Institute-Common Terminology Criteria for Adverse Events National Cancer Institute-Common Toxicity Criteria
ORRNCI-CTCAE	Objective Response Rate National Cancer Institute-Common Terminology Criteria for Adverse Events
OSORR	Overall Survival Objective Response Rate
PDOS	Progressive disease or Pharmacodynamic Overall Survival
PFSPD	Progression Free Survival Progressive Disease or Pharmacodynamic
PSPFS	Performance Status Progression Free Survival
PRPS	Partial Response Performance Status
RECISTPR	Response Evaluation Criteria in Solid Tumors Partial Response
SDRECIST	Stable Disease Response Evaluation Criteria in Solid Tumors
SFSD	Shortening Fraction Stable Disease
TNMSF	primary tumor/regional lymph nodes/distant metastasis Shortening Fraction
TTPTNM	Time to Tumor Progression primary tumor/regional lymph nodes/distant metastasis
TTP	Time to Tumor Progression

SCHEMA

Treatment	
Days 1, 8, 15	Ganetespib IV over 1 hour
Days 1-28	Sirolimus once daily
Day 28	End of Cycle

Patients will receive 28 day cycles of Ganetespib + Sirolimus until disease progression or unacceptable toxicity for up to 1 year (13 cycles).

Response evaluations (using WHO criteria) will be performed after every 2 treatment cycles (prior to each odd cycle 3, 5, 7, etc.).

Synopsis

Primary Objective

1. Phase I: To assess the safety, tolerability, and maximum tolerated/ recommended dose of ganetespib when administered in combination with sirolimus in patients with refractory sarcomas or unresectable or metastatic sporadic or neurofibromatosis type 1 (NF1) associated MPNST.
2. Phase II: To determine the clinical benefit of ganetespib in combination with sirolimus for patients with unresectable or metastatic sporadic or neurofibromatosis type 1 (NF1) associated MPNST.

Secondary Objectives

1. Phase I: To describe the plasma pharmacokinetic profile of ganetespib and sirolimus when administered in combination therapy
2. Phase I/II: To determine changes in pharmacodynamic parameters including phospho-S6, phosphorylated eIF2 alpha, Akt Phosphorylation, Hsp70, and G6PD in tumor tissue and peripheral blood mononuclear cells at baseline and during treatment and correlate with changes in clinical or radiologic outcome.
3. Phase I/II: To assess patient-reported pain severity and the impact of pain on daily activities before and during treatment with ganetespib and sirolimus and to correlate with changes in clinical or radiologic outcome.
4. Phase I/II: To evaluate the utility of three-dimensional MRI (3D-MRI) analysis in comparison to 1-dimensional and 2-dimensional measurements as a method to more sensitively monitor response.

Hypothesis and Rationale

Previously, no targeted agents have been able to cause tumor regression in a genetically engineered MPNST mouse model or human MPNST. Recently published data from the Cichowski laboratory demonstrated using Hsp90 inhibitors to enhance endoplasmic reticulum stress coupled with the mTOR inhibitor sirolimus led to dramatic tumor shrinkage in a transgenic MPNST mouse model, which correlated with profound damage to the endoplasmic reticulum and cell death. Ganetespib is a novel, injectable, small molecule inhibitor of Hsp90 and is currently being investigated in adults with a broad range of tumor types with a favorable safety profile and promising early results. Ganetespib has been studied in preclinical *in vivo* models with a variety of targeted agents with no marked apparent pharmacological interactions. Sirolimus is a commercially available orally administered mTOR inhibitor and is the active metabolite of temsirolimus, which is FDA approved agent for advanced metastatic renal cell carcinoma. Sirolimus has been studied and tolerated in combination with multiple cytotoxic and targeted agents in a variety of tumor types. Based on strong preclinical rationale, we hypothesize that ganetespib in combination with sirolimus will cause tumor regression in patients with refractory MPNSTs.

Trial Design

We propose a multi-institutional open label phase I/II trial of ganetespib in combination with sirolimus in patients with refractory sarcoma including MPNST. The primary objective of the initial component is to determine the safety, tolerability and recommended dose of this novel combination in a limited dose escalation phase I trial. Hsp90 inhibitors and mTOR inhibitors have also both demonstrated benefit in a variety of preclinical bone and soft tissue sarcoma models. We hypothesize that these agents that work on separate and potentially synergistic pathways will also be beneficial for other refractory bone and soft tissue sarcomas. Thus, the phase I component will be open to patients with refractory sarcomas, which will also expedite enrollment. Ganetespib will be given intravenously over one hour on days 1, 8, and 15 every 28 days. Sirolimus will be given orally daily continuously (28 days = 1 cycle). Upon determination of the recommended dosing, the primary objectives of the phase II portion will be to determine the clinical benefit rate (CR, PR, or stable disease ≥ 4 months using WHO criteria) of ganetespib in combination with sirolimus for patients with refractory MPNST. Secondary objectives include determination of the pharmacokinetic profile of these agents in combination and pharmacodynamic markers in tumor tissue and peripheral blood mononuclear cells, patient reported pain outcomes, and volumetric MRI analysis of tumor measurement.

Maximum Total Number of Subjects

Phase I: 3 to 6 patients per cohort with 1 dose escalations (potential for 2 de-escalations). Thus a minimum of 6 patients to a maximum of 18 patients are required.
Phase II: 10 patients in first stage with an additional 10 patients in the second stage for a total of 20 patients. The maximum number of evaluable patients for entire study will be 38.

Target Population

Individuals ≥ 16 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. Phase I component will also be open to patients with other refractory or relapsed sarcomas.

Anticipated Length of Study (patient enrollment period, overall length including follow-up)

Patients will be able to remain on treatment for up to 1 year (13 cycles) as long as they do not experience progressive disease or unacceptable toxicity. It is expected that 15-25 patients will be enrolled per year, and enrollment is expected to be completed in approximately 2.5 years

Study Drug (s)

Ganetespib intravenous
Sirolimus 2 mg oral tablets

Dosing and Administration

Ganetespib will be administered intravenously over 1 hour on days 1, 8, 15 every 28 days
Sirolimus will be administered once daily continuously
1 cycle = 28 days

Efficacy Evaluations

Response evaluations (WHO) with appropriate imaging studies (MRI/CT) will be performed at baseline and prior to odd cycles (3, 5, 7, etc.).

Safety Evaluations/Concerns

History and physical examinations and laboratory evaluations will be routinely performed during treatment study. For the phase I component: The recommended doses will be based on toxicities observed over the first treatment cycle. Dose modifications and management plans are specified in the protocol.

Correlative Studies

- Pharmacokinetic samples will be collected in all phase I patients (mandatory) and in upwards of 10 patients in the phase II component for data and experience at the recommended dose. Detailed pharmacokinetic sampling will occur at steady state during cycle 1.
- Correlative studies evaluating pharmacodynamic parameters on Hsp inhibition (Hsp70), mTOR inhibition (phospho-S6 and Akt Phosphorylation), UPR activation (EIF2α phosphorylation), and oxidative stress (G6PD) will be explored in tumor tissue and peripheral blood mononuclear cells at baseline and during treatment.
- 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 Brief Pain Inventory will be used to assess the impact of pain on daily activities. These tests will be given prior to treatment and then prior to cycle 3, 5, 9, and 13 when disease evaluation is performed.

Brief Statistical Design

In the phase I component, a conventional 3+3 dose-escalation design is used. The initial starting dose of ganetespib is 150 mg/m^2, approximately 1 dose level below the recommended phase 2 weekly dose, in combination with the recommended adult dose of sirolimus of 4 mg once daily. This will be followed by one dose escalation of the ganetespib to 200 mg/m^2 weekly (recommended phase 2 dose) and sirolimus recommended adult dose. The Maximum tolerated dose (MTD)/Recommended dose will be defined as the dose level immediately below the level at which $\geq$ 33% of patients in a cohort experience a dose-limiting toxicity (DLT) based on toxicities observed in the first treatment cycle.

In the phase II component, the primary endpoint will be clinical benefit rate, which will be defined as a CR, PR, or stable disease $\geq$ 4 cycles. An evaluable patient will be classified as a responder (success) for the primary endpoint if the patient achieves a PR, CR or stable disease at $\geq$ 4 months. The target clinical benefit rate will be 25%, and a clinical benefit rate $\leq$ 5% will be considered uninteresting. Using a Simon's optimal two-stage phase II design, the first stage will require 10 patients, with no further accrual if 0 of 10 respond. If $\geq$ 1/10 patients respond, accrual will continue until a total of 20 patients have been enrolled. If $\geq$ 3/20 patients respond, this

combination will be considered of sufficient activity. Assuming the number of successes is binomially distributed, this design has a one sided alpha of 0.07 and a power of 88% for detecting a true success probability of at least 25% versus the null hypothesis success rate of 5% or less.

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Primary Objective

1. Phase I: To assess the safety, tolerability, and maximum tolerated dose (MTD)/ recommended dose of ganetespib when administered in combination with sirolimus in patients with refractory or relapsed sarcomas including unresectable or metastatic sporadic or neurofibromatosis type 1 (NF1) associated MPNST.
2. Phase II: To determine the clinical benefit of ganetespib in combination with sirolimus for patients with unresectable or metastatic sporadic or NF1 associated MPNST.

1.2. Secondary Objectives

1. Phase I: To describe the plasma pharmacokinetic profile of ganetespib and sirolimus when administered in combination therapy
2. Phase I/II: To determine changes in pharmacodynamic parameters including phospho-S6, phosphorylated eIF2 alpha, Akt Phosphorylation, Hsp70, and G6PD in tumor tissue and peripheral blood mononuclear cells at baseline and during treatment and correlate with changes in clinical or radiologic outcome.
3. Phase I/II: To assess patient-reported pain severity and the impact of pain on daily activities before and during treatment with ganetespib and sirolimus and to correlate with changes in clinical or radiologic outcome.
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2. BACKGROUND

2.1 Malignant peripheral nerve sheath tumors

Malignant peripheral nerve sheath tumors (MPNSTs) are soft tissue sarcomas arising from peripheral nerve or show nerve sheath differentiation and are associated with a high risk of local recurrence and metastasis [1]. MPNSTs account for 10% of all soft tissue sarcomas, and carry the highest risk for sarcoma specific death among all the soft tissue sarcoma histologies [2]. At present, complete surgical resection is the only curative treatment for MPNST [3,4]. MPNSTs are at high risk for local recurrence (32-65%) [5] and metastasis (40%) [6]. The most frequent sites of metastasis of MPNSTs are lung, liver, brain, soft tissue, bone, lymph nodes, and retroperitoneum [7]. The outcome for unresectable, recurrent, or metastatic MPNST is dismal.

Neurofibromatosis Type 1 and MPNST

Approximately half of all MPNSTs arise from individuals with NF1 [8]. NF1 is a common autosomal dominant tumor predisposition syndrome. The gene responsible for NF1 encodes for a protein called neurofibromin, which includes a GTPase activating protein that regulates hydrolysis of Ras-GTP to Ras-GDP [9,10]. Patients with NF1 have decreased levels of

neurofibromin, which can lead to dysregulated Ras and tumorigenesis [11]. 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 [7,8]. The majority of NF1-associated MPNSTs arise within pre-existing plexiform neurofibromas [12]. This may lead to difficulty and delay in diagnosing MPNSTs in patients with NF1 because the clinical indicators of malignancy such as mass, pain, and edema may also be features of active, benign plexiform neurofibromas. NF1 associated MPNSTs are frequently more located in the trunk [13,14] as opposed to extremity location seen more commonly in sporadic MPNSTs, tend to be large (>5cm) [15], and may have a greater propensity to metastasize [7]. These may be potential reasons why NF1-assoicated MPNSTs appear to have a worse outcome than sporadic MPNSTs. Gene expression profiling of NF1 associated (n=25) and sporadic (n=17) MPNSTs did not identify a molecular signature that could reliably distinguish between both groups [16,17].

Treatment

The only known curative approach to MPNSTs is complete surgical resection with wide negative margins [3,4]. Thus, early diagnosis of MPNST is crucial, which may be difficult in patients with NF1 for the reasons stated above. Radiotherapy is used in situations where the sarcoma is not amenable to surgical resection, but extremely high doses are needed, and the local control rate is only 30-60%. Clinical trials have shown that external beam radiation or brachytherapy in addition to limb sparing surgery can improve local control in patients with soft tissue sarcomas [18,19]. Thus adjuvant radiotherapy is recommended to improve local control in intermediate and high grade lesions > 5 cm after a marginal excision [1,19]. The role of chemotherapy for MPNSTs has not been defined to date. In retrospective reviews, NF1 associated MPNSTs have been described to have much lower response to chemotherapy than sporadic MPNSTs [3,15].

There is a current DoD sponsored, SARC coordinated phase II clinical trial evaluating the response rate of high grade unresectable MPNST to standard chemotherapy agents (Doxorubicin, Ifosfamide, Etoposide) (SARC006). The primary trial endpoint (CR and PR rate) is determined after 4 cycles of chemotherapy (2 cycles of IA followed by 2 cycles of IE). Local control with surgery and or radiation follows cycle 4, and the administration of 2 additional cycles IE and IA each. Patients are stratified for the presence of a sporadic vs. NF1 associated MPNST, and a two-stage phase II design is used targeting a response rate ≥ 40% (rule out < 20%). Seventeen patients are enrolled on the initial stage, and enrollment in a stratum is expanded if ≥ 4/17 respond after 4 cycles of chemotherapy. In the NF1 MPNST stratum 4 partial responses were observed in the first 17 patients enrolled, and enrollment was thus expanded. To date (February 2013), 33 patients with NF1 MPNST have been enrolled. Of these, 29 patients are currently evaluable for response. Five patients had a partial response as best response, 21 stable disease, and 3 progressive disease (Objective Response (OR) rate 17.2%. Fifteen patients with sporadic MPNST have been enrolled. Of 12 patients currently evaluable for response, 4 partial responses, 6 stable disease and 2 progressive disease have been observed (OR rate 33.3%). The study was closed before full enrollment due to slow accrual. While the primary trial objective was not reached, with only 5/29 ORs in the NF1 stratum, the desired OR rate of 11+/37 would have unlikely been met even if accrual had been completed. A lower OR rate in NF1 compared to sporadic MPNSTs was

observed similar to retrospective literature reports. In addition to PRs after both IA and IE, disease stabilization was achieved in most patients.

Based on the high incidence of MPNST in NF1, limited treatment options, and high mortality, there is clearly a desperate need for more effective medical treatments for MPNSTs. With increasing understanding in the molecular pathogenesis of MPNSTs, clinical trials with targeted agents have become available. Several histology specific trials with targeted agents have been performed for MPNSTs (Table 1). A phase II trial of the EGFR inhibitor erlotinib was the first histology specific trial with a targeted agent to be completed in patients with NF1 and sporadic MPNST. Twenty-four patients were enrolled within 22 months in 13 institutions. Although this trial and other completed trials demonstrate lack of activity and rapid disease progression with a median time to progression of < 2.0 months in MPNST, they did demonstrate that timely completion of specific trials in this rare malignancy is feasibly, and novel therapies are warranted in this aggressive disease.

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[20]	19/20 pts. Progressive disease at 2 months 1 stable disease	[21]
Sorafenib	C-Raf, B-Raf, VEGFR2, C-Kit, PDGFR	II	12	Oral continuous	Response RECIST[22]	No responses; median progression free survival 1.7 months	[23]
Imatinib	C-Kit, PDGFR, VEGFR	II	7	Oral continuous	Response RECIST[22]	No responses; 1 stable disease	[24]
Dasatinib	C-Kit, SRC	II	14	Oral continuous	Response Choi[25]	No response or stable disease	[26]
Bevacizumab/ RAD001	Angiogenesis /mTOR	II	-	IV q14d / oral continuous	Response WHO[20]	Currently ongoing	--

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 MPNSTs. Mammalian target of rapamycin (mTOR) has been reported to be hyperactivated in NF1-deficient tumors as a consequence of aberrant Ras signaling. Using an *Nf1/p53*-mutant MPNST model, the Cichowski laboratory demonstrated that sirolimus, an mTOR inhibitor, suppressed tumor growth in potent, but cytostatic effect [27]. The MPNST transgenic mouse model tumors ultimately became resistant to treatment with sirolimus, which was associated with re-vascularization and upregulation of vascular endothelial growth factor (VEGF). Based on this data, we collaboratively developed a phase II trial of RAD001 and bevacizumab for refractory MPNST. The primary trial objectives are to determine the clinical benefit rate (CR, PR, and SD at ≥ 4 months using WHO criteria) of this treatment. The consistently rapid disease progression justifies inclusion of SD ≥4 months as clinical

benefit. Secondary objectives are to evaluate the spectrum of germline NF1 mutations, and to analyze the PD of RAD001 in peripheral blood specimens including p70 S6 kinase 1, eIF4E, eIF2 alpha VEGF, VEGFR, and Akt phosphorylation. RAD001 is administered at a dose of 10 mg orally once daily on a continuous dosing schedule (28 days = 1 cycle). Bevacizumab is administered IV at a dose of 10 mg/kg/dose every 14 days. A two-stage design is used with a target clinical benefit rate of 25%. This trial will be coordinated by SARC, and SARC and NF Consortium sites will participate. Novartis provides RAD001 and Genentech bevacizumab. The DoD is providing support to this trial through a Clinical Trial Award. The trial opened for enrollment in December 2012.

Building on this study, and identifying alternative targets in combination with mTOR may be beneficial. Proteotoxic or endoplasmic reticulum (ER) stress is induced when unfolded proteins accumulate in the ER [28]. Oncogenic RAS also causes ER stress [29], and when ER stress level become insurmountable, cell death ensues, suggesting agents that enhance ER stress may be developed as anti-cancer agents. In an exciting new development, the Cichowski laboratory recently demonstrated that further enhancing ER stress using HSP90 inhibitors coupled with sirolimus led to dramatic tumor shrinkage in the transgenic mouse model, which correlated with profound damage to the ER and cell death [30]. Previously, no targeted agents have been able to cause tumor regression in a genetically engineered models or human MPNST trials (Table 1).

2.2 Refractory bone and soft tissue sarcomas

The prognosis in advanced and metastatic sarcomas remains poor. Continued progress while minimizing acute and late effects of current therapy is dependent on the development of novel therapeutic approaches for malignant sarcomas. Hsp90 inhibitors have demonstrated benefit in a variety preclinical of bone and soft tissue sarcomas, including synovial sarcoma[31,32] Ewing Sarcoma [33], osteosarcoma [34,35], and rhabdomyosarcoma [36,37]. Disruptions of the PI3K-Akt-mTOR signaling pathway are associated with different sarcoma types[38]. Rapamycin and other mTOR inhibitors have demonstrated inhibition of growth in a variety of *in vitro* and *in vivo* sarcoma preclinical models [39-43]. Early clinical studies of single agent mTOR inhibition have demonstrated potential benefit in a subset of sarcoma patients who have failed standard therapy (variety of soft tissue sarcoma, GIST, Ewing Sarcoma, and Osteosarcoma), however the activity of this drug has been primarily cytostatic [38], and activation of feedback mechanisms have been of concern [44]. Thus rationale combination of agents with mTOR inhibitors may result in improved responses in these difficult to treat patients. mTOR signaling has also been implicated in ER stress pathway [45]. We hypothesize that ganetespib and rapamycin, two agents that work on separate and synergistic pathways, will not only be beneficial to MPNSTs, but other refractory bone and soft tissue sarcomas. Thus, we will also include other sarcoma types to the phase I component of this trial.

2.3 Study Agents: Ganetespib and Sirolimus

Ganetespib

Ganetespib (formerly called STA-9090) is a novel, injectable resorcinolic triazolone small molecule inhibitor of Hsp90 [46]. Hsp90 is a molecular chaperone that regulates posttranslational folding, stability, and function of its client proteins, many of which play

critical roles in cell growth, differentiation, and survival [47,48]. Ganetespib inhibits Hsp90 chaperone activity by binding to its N-terminal adenosine triphosphate (ATP) pocket. Hsp90 inhibition causes its client proteins to adopt aberrant conformations, which are then targeted for ubiquination and degradation by the proteosome [49]. Hsp90 client proteins include wild type and mutated forms of many important signaling proteins associated with cancer, such as BCR-ABL, BRAF, CDK4, KIT, c-MET, c-SRC, EGFR, LCK, HER2 and VEGFR. Ganetespib is currently being investigated in adults with a broad range of tumor types. It is being explored in both once weekly and twice weekly schedule, and has been generally well tolerated. Most frequent adverse events were gastrointestinal or constitutional in nature, and were generally mild to moderate in severity. Preliminary signals of anti-tumor activity have been observed on the ongoing clinical trials in a broad range of tumor types.

Preclinical studies

Anti-tumor in vitro and in vivo studies:

Detailed descriptions are provided in the investigator's brochure and results are highlighted here [50]. Ganetespib is a potent inhibitor of cell death in a broad range of hematologic and solid tumor cell lines including those expressing mutant Hsp90 client proteins with IC_{50} in the low nM concentrations. Ganetespib was also able to induce the degradation of various client proteins, including those that are mutated, amplified, or rearranged. In a KRAS mutant NSCLC cell line, ganetespib effectively destabilizes the KRAS effector CRAF and blocks both MAPK and AKT signaling. Ganetespib has demonstrated in vivo efficacy in > 13 human tumor xenograft models including a broad spectrum of tumor histologies, and response appeared to be dose responsive in some cell lines.

Animal Toxicology:

A comprehensive, non-clinical program has been conducted to characterize the toxicological and toxokinetic profile of ganetespib. Detailed descriptions of all these studies are provided in the investigator's brochure and highlighted here. Rats survived a single 30 mg/kg dose of ganetespib, but doses of 85 or 250 mg/kg elicited morbidity and mortality. For rats given 100 mg/kg (reduced to 85 mg/kg beginning with the third dose) and assigned to a 14-day recovery evaluation, most changes returned to normal or improved. Rats given 10, 30, 75, or 100 mg/kg once weekly for 4 weeks had no abnormal clinical findings. The maximum tolerated single-administration dose in cynomolgus monkeys was 11 mg/kg. When administered on 2 consecutive days, up to 3 mg/kg/day was well tolerated. In 3-month studies with ganetespib administered on Days 1 and 15 of four 21-day cycles, rats tolerated 20 mg/kg/dose of ganetespib, the NOAEL. Transient decreases in weight gain occurred at 50 and 100 mg/kg/dose, and early deaths occurred at 100 mg/kg/dose. In another 3-month study, cynomolgus monkeys on that regimen tolerated doses up to 7 mg/kg/dose, the NOAEL. Transient diarrhea occurred at 2, 4, and 7 mg/kg/dose, with reversible microscopic pathologic changes occurring at 7 mg/kg/dose. Ganetespib was considered to be well tolerated by cynomolgus monkeys when administered by 1-hour infusion twice weekly for 4 weeks via an implanted silicone venous catheter. When pregnant female rats were given ganetespib daily by infusion during organogenesis, maternal toxicity (clinical signs, decreased weight gain and food consumption) and developmental toxicity (post-implantation loss) occurred at doses of 3 mg/kg/day and higher. Ganetespib was rapidly eliminated from

retinal tissue and did not cause photoreceptor cell apoptosis. Unlike 17-DMAG and AUY922, ganetespib was not associated with ocular toxicity in the rat model.

Preclinical pharmacology
Studies evaluating the distribution, biotransformation, and elimination, ganetespib exposure increased dose proportionally. Ganetespib is highly protein bound (98.6-98.7% in human plasma) and highly distributed throughout tissue with the exception of the central nervous system. Mean terminal half-life ($t_{1/2}$) for ganetespib after multiple doses were approximately 6 and 11 hours in rates and monkeys respectively. Ganetespib was extensively metabolized in the liver to mainly glucoronide conjugates. Ganetespib is excreted through the feces, the major route of excretion. Ganetespib does not appear to accumulate after multiple dosing. Fecal elimination via bile is the major route of excretion. Ganetespib is an inhibitor of CYPC19 and CYP3A4 (midazolam specific) in human liver microsomal systems but does not markedly inhibit transporters including P-gp. Ganetespib does not appear to be an inducer of CYP or UGT enzymes. In mice, ganetespib did not show marked pharmacokinetic interactions when co-administered with paclitaxel, docetaxel, erlotinib, fulvestrant, BEZ235, AZD6244, bortezomib, and irinotecan.

Clinical experience
Clinical studies
As of September 2014, approximately 1362 subjects have received ganetespib. Overall, ganetespib has been well tolerated with most reported adverse events (AEs) being mild to moderate in severity. The most frequently reported AEs in the largest pooled data set (single agent studies, n=402) are related to gastrointestinal (GI) toxicity, including diarrhea (79%), nausea (44%), decreased appetite (31%), vomiting (27%), constipation (22%), and abdominal pain (20%). Non-GI related toxicity includes fatigue (53%), headache (20%), and anemia (21%). In the Phase 2b study in patients treated with ganetespib in combination with docetaxel, preliminary findings show similar safety profile with 53% reduction in the incidence of diarrhea due to implementation of anti-diarrheal prophylactic medicine.

Ocular toxicity, manifested as visual disturbances, has been reported for several Hsp90 inhibitors. Of the 601 patients treated with ganetespib (single and combination studies), 6 (1%) of patients experienced event of blurred vision and 4 (< 1%) experienced visual impairment that was assessed as related. The studies using single agent ganetespib (n=378), 4 patients (1%) experienced treatment related blurred vision and 3 patients (< 1%) experienced treatment related visual impairment. In studies using single agent ganetespib, visual disturbances regardless of relationship to treatment included blurred vision (5%), visual impairment (2%), vitreous floaters, cataract, conjunctival hemorrhage, conjunctivitis, dry eye, eyelid edema, periorbital edema, reduced visual acuity, chromatopsia, conjunctival hyperemia, eye swelling, eyelid ptosis, glaucoma, night blindness, ocular hyperemia, photopsia, and scotoma (all < 1%).

The mechanism of visual disturbances is linked to induction of apoptosis in cells in the outer nuclear layer of the retina, which occurs following treatment with 17-DMAG or AUY922 [51]. In contrast, ganetespib did not elicit induction of apoptosis in preclinical studies using rodent

models, consistent with the very low number of reported visual disturbance cases in the clinic.

Hepatocellular injuries are usually detected by enzyme elevations in serum aminotransferases (ATs), total bilirubin, and alkaline phosphatase. In the combination treatment arm of Study 9090-08, AEs of elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were reported in 3% and 2%, of patients, respectively. An AE of elevated alkaline phosphatase was reported in 3% of patients and an AE of elevated bilirubin was reported in < 1% of patients. Grade 3 elevations of AST and ALT were reported in < 1% and 2% of patients, respectively. In the pooled data from 322 patients who received single-agent ganetespib, AEs of elevated AST and ALT were reported in 14% of patients. An AE of elevated alkaline phosphatase was reported in 16% of these patients and an AE of elevated bilirubin was reported in 6%. Grade ≥ 3 elevations of AST and alkaline phosphatase were reported in 4% of patients and Grade ≥ 3 elevations of ALT and bilirubin in 3% of patients. None of the patients reported concomitant elevations of ATs ≥ 3X the upper limit of normal (ULN) and bilirubin ≥ 2X ULN.

Liver toxicity in the 1st-generation geldanamycin-derivative Hsp90 inhibitors is an off-target effect. According to a study by Cysyk et al, the presence of benzoquinone moiety in the molecule is the suspected cause of liver toxicity [Cysyk et al, Chem Res Toxicol, 2006]. Ganetespib does not contain the benzoquinone moiety and, therefore, liver toxicity is not expected. This correlates with the safety information collected to date.

Cardiac Findings: A thorough QT study examining ECG intervals and morphology was conducted in accordance with ICH E14. The study was conducted as a randomized partially double blind, placebo and positive controlled, 3-arm, crossover study to assess the effect of ganetespib on ECG parameters in healthy volunteers. Volunteers were administered a single dose of 200 mg/m^2 ganetespib. Analyses of ECG data revealed a placebo-corrected modest change in QTcF from baseline of 21.5 msec at 24 hours post-dose. No increase of QTcF was observed at the time of ganetespib Cmax (at end of infusion). QTcF was back to baseline 7 days after the ganetespib dose. To date in the clinical development program, there have been 362 patients treated with ganetespib as a single agent and 218 treated with ganetespib with docetaxel (total 580 patients). Seven patients (1.2%) had prolonged QT interval reported as AE and none had torsades de pointes or other ventricular arrhythimias on any ECG recording. Eight potential deaths resulting from cardiovascular SAEs have been reported and were described by investigators as cardiac arrest (n=3), sudden cardiac death (n=2), cardiopulmonary failure (n=1), and cardiovascular insufficiency (n=1). The incidence of deaths on ganetespib due to cardiovascular SAEs does not seem excessive for this advanced cancer population. While the effect of ganetespib on QT interval is being completely characterized, we have added recommended provisions to eligibility criteria for the cardiovascular system and included interval ECG monitoring for all patients on study.

In phase I solid tumor trials, the doses have ranged from 7 to 259 mg/m^2 once weekly and 2 to 200 mg/m^2 twice weekly. The MTD for once weekly dosing in solid tumors was established to be 216 mg/m^2 based on DLTs of asthenia and diarrhea. The recommended

single agent dose is 200 mg/m^2 once weekly x 3, one week off. For the twice weekly dosing, the recommended dose is 150 mg/m^2 (Monday/Thursday) x 3, one week off. In hematological malignancies, the single agent once weekly is 200 mg/m^2 and 90 mg/m^2 twice weekly with no week off for either schedule.

Ongoing phase 2 studies are evaluating the activity of ganetespib in a variety of solid tumors including HCC, colorectal cancer, lung cancer, melanoma, prostate, pancreatic, and breast.

A phase I study of ganetespib in combination with docetaxel has completed enrollment. The GI AEs continued to be observed with ganetespib and hematologic AEs are primarily seen with docetaxel. Following the evaluation of DLTs and overall tolerability, the recommended combination dose is 150 mg/m^2 of ganetespib on days 1 and 15 in combination with 75 mg/m^2 of docetaxel on day 1 in a 21 day cycle. The current phase 2 randomized phase 2B/3 looking at this combination in comparison to docetaxel alone in NSCLC is ongoing. Interim results show promising signals of activity in pre-specified populations. This combination appears to be well-tolerated in this patient population.

Pharmacokinetics and Correlative Biology Studies
The pharmacokinetics of ganetespib administered at various doses on a weekly or twice weekly schedule are under investigation. Ganetespib pharmacokinetics appears to be linear and dose proportional. The mean terminal half-lives have ranged from approximately 5 to 15 hours. Ganetespib plasma concentrations following the first and subsequent doses are comparable following either once or twice-weekly dosing, indicating the lack of drug accumulation. Ganetespib plasma concentrations are also comparable in the solid and hematologic tumor patients. C_{max} and AUC increase in approximate proportion to dose irrespective of dosing day with virtually identical dose-exposure ratios for doses given on different days, indicating linear PK (r^2 = 0.7080 and 0. 7596 for C_{max} and AUC versus dose, respectively). Ganetespib C_{max} correlates well with AUC (r^2 = 0.9338). CL and V_d are approximately constant across doses.

Hsp70 was quantified from RNA that was purified from blood cell pellets derived from subjects in a phase I trial (protocol 9090-02) at multiple time points on the first day of treatment hours. Peak maximum induction occurred at 2 hours post start of infusion. Additional genes were also investigated to supplement RNA analysis in order to monitor downstream targets of Hsp90 client proteins. The Janus kinase (JAK) signal transducer and activator of transcription (STAT) signaling pathway is disrupted by drug treatment, and results in downstream regulation of the genes controlled by the transcription factor STAT3.

Sirolimus
Sirolimus (rapamycin) is a mammalian target of rapamycin (mTOR) kinase inhibitor that has been FDA approved for immunosuppression following kidney transplantation [52]. Sirolimus inhibited 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 [53,54]. Rapamycin analogs, such as temsirolimus [55], an intravenous soluble ester (pro drug) of sirolimus and everolimus [56], 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 the others in a particular tumor type.

Preclinical studies

Preclinical studies have demonstrated efficacy of sirolimus analogs and parent compound in multiple tumor types. In the NCI 60 tumor cell line panel, mTOR inhibitors demonstrated growth inhibitory activity against a broad spectrum of tumors including leukemia, brain, renal, breast and melanoma [57,58] with an average IC_{50} of 8.2 nM. Subsequent xenograft studies have confirmed the cytostatic properties of the mTOR inhibitors.

Clinical studies in malignancy

A pharmacodynamic continuous reassessment method-based phase I study of rapamycin in adult patients with solid tumors was performed [59]. 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 [60]. 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 [61]. 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 [62] and chemoradiation in NSCLC [63] 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.

2.4 Rationale

mTOR and NF1

Signaling intermediates downstream of Ras are hyperactivated as a result of *NF1* gene inactivation and these specific proteins are critical for transmitting the Ras growth signal and for the development of neoplasia in patients with NF1 [11]. One of these downstream proteins is the mTOR molecule. Studies have demonstrated that the *NF1* tumor suppressor regulates mTOR pathway activation. mTOR was found to be activated in both NF1 deficient primary human and mouse cells as well as in human and genetically-engineered *Nf1* mouse tumor models. This aberrant activation was dependent on Ras and PI3 kinase/AKT signaling [64,65]. In this regard, *Nf1* loss in mouse embryonic fibroblasts [65] and primary mouse astrocytes [64] was shown to result in Ras- and PI3K-dependent mTOR pathway activation, which could be inhibited with sirolimus. Moreover, *Nf1-/-* astrocytes are highly sensitive to sirolimus treatments that have no effect on normal astrocyte growth [64]. The increased proliferation associated with loss of neurofibromin expression in human MPNST cell lines was dramatically reduced by treatment with sirolimus [65]. Sirolimus treatment of optic gliomas developing in a genetically-engineered *Nf1* mouse model resulted in attenuated mTOR signaling *in vivo* [58]as well as tumor growth *in vivo* [66]. While tumors rapidly ceased to proliferate, there was no evidence of apoptosis or senescence, and sirolimus had no early effect on microvasculature in either preclinical model.

mTOR and MPNST

A transgenic mouse model [67] carrying compound mutations in the *Nf1* and *p53* tumor suppressors on the same chromosome develop aggressive MPNSTs that are histologically indistinguishable from human MPNSTs. Tumors grow with consistent and rapid kinetics, and on average mice only survive 10.7 days after the tumor is detected. This model was used to test the role of mTOR in tumorigenesis *in vivo* and assess the therapeutic utility of rapamycin [27]. Animals with palpable tumors (approximately 300 mm^3) were injected I.P. with 5 mg/kg rapamycin per day. Control mice died on average in 12.2 days, and tumors grew 9.7-fold. In contrast, rapamycin potently suppressed MPNST growth, and allowed the animals to survive. Inhibition of S6 phosphorylation was observed in tumor and non-tumor tissue, demonstrating that rapamycin was effectively suppressing the mTOR pathway *in vivo*. Moreover rapamycin mediated its anti-tumor effects within 24 hours by potently suppressing proliferation, as assessed by BrDU incorporation in control and rapamycin treated tumors. Consistent with *in vitro* observations, apoptotic and senescent cells were not detected. As such, tumor growth was dependent on continued exposure to rapamycin, as tumors re-exhibited S6 phosphorylation and resumed growing at a rate comparable to control treated tumors following rapamycin removal. Although the response to rapamycin was potent, effects were cytostatic. Therefore, Dr. Cichowski and her lab have been using this model to develop more effective mTOR-inhibitor based combination therapies [30]. To identify

additional therapeutic targets, her group considered drugs that might exploit cellular vulnerabilities of cancer cells.

Hsp90 and mTOR combination

Tumor cells often exhibit specific stress-related phenotypes caused by insults such as excessive DNA damage as well as replicative, metabolic, and proteotoxic stress [68]. Agents that further enhance or sensitize cancer cells to these stresses could thus be developed as potential anti-cancer therapies [69,70]. Proteotoxic or endoplasmic reticulum (ER) stress is induced when unfolded proteins accumulate in the ER [28]. Cancer cells frequently exhibit high levels of ER stress caused by a multitude of factors [69,70]. Oncogenic RAS also causes ER stress [29]. Aneuploidy in particular has been shown to induce proteotoxic stress in cells [71]. ER stress activates a signal transduction pathway called the unfolded protein response (UPR) [28]. The UPR is an initially protective mechanism to reduce protein accumulation, however, when ER stress levels become insurmountable, cell death ensues [28]. Hsp90 maintains protein homeostasis by folding newly synthesized and misfolded proteins, assembling and dissembling protein complexes and resolving protein aggregates [47]. Hsp90 also directly stabilizes two key stress sensing components of UPR: IRE1 and pPERK/PERK [72]. Therefore Hsp90 inhibitors would expect to promote ER stress in cancer cells directly by impairing global protein folding in already compromised tumor cells and inactivating subsequent adaptive responses of UPR. Under these observations, the Cichowski group hypothesized the therapeutic benefits of Hsp90 inhibitors alone and in combination with mTOR inhibition for Ras and/or mTOR driven tumors [30]. The following summarizes the exciting new discovery recently published [30].

MPNSTs are highly aneuploid and are driven by constitutive activation of Ras. They were demonstrated to have much higher levels of ER stress when compared to normal peripheral nerve as confirmed by three markers of UPR activation (BiP upregulation, eukaryotic translational initiation factor 2α (eIF2α) phosphorylation, and accumulation of the spliced active form of XBP-1 (sXBP-1). In addition, agents that induced ER stress triggered cell death at concentrations that did not affect the viability of normal cells.

The Hsp90 inhibitor used in this pre-clinical model was IPI-504. However, two additional structural distinct Hsp90 inhibitors BEP800, AUY-922, and 17-AAG killed MPNSTs, induced ER stress, and impacted UPR with the same kinetics IPI-504, confirming that these agents all function by suppressing Hsp90 [30]. In addition, subsequent studies using ganetespib demonstrated similar results (Personal Communication, Karen Cichowski). IPI-504 was administered at a dose of 100 mg/kg once weekly, and rapamycin was administered at 5 mg/kg daily. Alone, IPI-504 was unable to promote tumor regression, but when combined with rapamycin, tumors shrank on average 49%. Tumor regression was visually apparent and histologic analysis revealed massive cell death and accumulating debris. Maximal tumor regression occurred with 3-5 days and no acute or long-term toxicity was observed.

IPI-504 rapidly induced ER stress and UPR activation and rapamycin sensitized MPNSTs to IPI-504. Rapamycin and IPI-504 trigger catastrophic destruction of the ER and mitochondria in MPNSTs as evidenced by ER swelling, destruction, and mitochondrial damage that was

seen in all tumors treated with both agents, but was not detected in tumors from animals exposed to single agents. This process is fueled by oxidative stress, which is caused by IPI-504 dependent production of reactive oxygen species, and rapamycin dependent suppression of glutathione, an important endogenous anti-oxidant. Similar tumor regression from this combination extended to a *KRAS* mutant mouse model of NSCLC.

To date, no previous targeted agents have been shown capable of causing tumor regression in the highly aggressive genetically engineered MPNST model or in human tumors (See Table 1). Pre-clinical data presented demonstrate strong scientific rationale to study this combination in a clinical trial. Ganetespib is a potent, next generation Hsp90 inhibitor that has shown superior activity to first generation agents in preclinical studies. It has a favorable safety profile and promising anti-tumor activity over a broad range of tumor types in early clinical trials. Sirolimus is commercially available, oral, and relatively inexpensive. It has a long safety record, demonstrated efficacy in preclinical cancer models, and was used in the transgenic MPNST mouse model. We propose a multi-institutional open label phase I/II trial of ganetespib in combination with sirolimus in patients with refractory MPNST. There is data to support this combination will benefit other sarcomas in addition to MPNST. Thus, we will include other sarcoma types to be eligible for the phase I component of this trial, which will also benefit in expediting enrollment. Pre-clinical work with this combination of agents in other sarcomas is currently underway, which will aid in the further development of this combination for other sarcomas. **The results of this trial will provide valuable toxicity, tolerability, and pharmacokinetic information for a drug combination with potential uses other Ras driven tumors.** By taking agents selected through this model to a clinical trial, we will be able to explore the utility of the mouse model for predicting response in NF1 clinical trials. Importantly, if the combination proves as effective as hypothesized in MPNSTs, it could provide a therapeutic strategy for this highly refractory and aggressive malignancy.

2.5 Preliminary results of current study (March 2015)

A total of 10 patients (9 evaluable) have enrolled on the phase I portion of this study. Three patients were enrolled on dose level 1. No DLTs were observed on the first dose level. On dose level 2, one out of 3 patients experienced a DLT of grade 4 thrombocytopenia and thus the cohort was expanded to an additional 3 patients (1 patient came off of protocol therapy due to physician decision prior to completing cycle one without experiencing a DLT and was thus replaced). The most common adverse effects were grade 1 or 2 diarrhea, grade 1 thrombocytopenia, and grade 1 increase in liver transaminases. No other DLTs were experienced, and dose level 2 was determined to be the recommended phase II dose to move forward.

Three out of 9 patients experienced a grade 1 or 2 infusion reaction. After being treated with steroids and diphenhydramine and in some cases slowing the infusion time to 2 hours, all patients recovered and were able to receive subsequent ganetespib with pre-medication without additional infusion reactions. Infusion reactions in general may occur in up to 10% of patients without pre-medication, and lower with pre-medication, which is generally being recommended across studies with ganetespib. Our study saw a slightly higher occurrence in

the small number of patients treated with this combination. Thus, we will require pre-medication prior to administration of ganetespib with steroids and anti-histamines for the phase II portion of the study.

2.6 Study Design

We propose an open label phase I/II trial of ganetespib in combination with sirolimus in patients with refractory sarcomas and MPNSTs. The primary objective of the initial phase I component is to evaluate the safety and tolerability of this combination, and to determine the maximum tolerated dose (MTD)/recommend dose for this combination. The phase I component will be open to all patients with refractory sarcomas. Secondary objectives of this portion will be to describe the plasma pharmacokinetic profile of ganetespib and sirolimus when administered in this combination therapy. The starting dose and escalation schema are as described below.

Dose Level	Ganetespib (mg/m^2) IV days 1, 8, 15 every 28 days	Sirolimus (mg)	
		Loading dose C1 D1 only	Maintenance PO once daily continuous
-2	100	6	2
-1	150	6	2
1*	150	12	4
2	200	12	4
* Starting dose			
Each cycle is considered 28 days			

The recommended phase II dose of weekly single agent ganetespib is 200 mg/m^2/dose IV over one hour x 3 weeks; 1 week off. The starting dose of Ganetespib selected is a 25% dose decrease and used previously in the docetaxel combination trials [49]. The starting dose for sirolimus was selected using similar doses derived from earlier phase I single and combination studies [59,61,62] with sirolimus trough levels ranging from 5-10 ng/mL. There will be no plans to exceed the recommended doses of either agent (Dose level 2). Three to six patients will be entered per dose level using standard 3+3 design. Patients will be closely evaluated for the development of toxicity. Toxicities observed during the first cycle will be used to define the MTD/Recommended dose.

The recommended phase II dose of this combination is dose level 2 with ganetespib 200 mg/m^2/dose IV given over one hour x 3 weeks (days 1, 8, 15), 1 week off combined with sirolimus 4 mg by mouth once daily continuously (loading dose of 12 mg given on day 1 of cycle 1 only). One cycle equals 28 days. The phase I portion is complete and the phase II component will open. The primary objective of the phase II portion will be to determine the clinical benefit rate (CR, PR, or stable disease ≥ 4 months using WHO criteria) of ganetespib in combination with sirolimus for patients with refractory sporadic or NF1 associated MPNST. Additional sarcoma strata may be considered directed by preclinical findings and rationale. We propose an open label Simon's optimal two-stage design with a target benefit rate of 25%.

Ten patients will be enrolled on the first stage, with no further accrual if no response (CR, PR, or stable disease ≥ 4 months) is observed. If at least one response is observe, accrual will continue until a total of 20 patients have been enrolled. If ≥ 3 of 20 evaluable patients responds, this regimen will be considered active and could provide a therapeutic upfront strategy for this malignancy. Sites of measureable disease will be evaluated within 4 weeks prior to starting therapy and then every 2 cycles. Patients will be able to remain on treatment as long as they do not experience progressive disease or unacceptable toxicity up to 13 cycles (1 year). If after 13 cycles, patients who in the opinion of the treating investigator are deriving benefit may be eligible for additional cycles, but will be determined at the discretion of the study PI, sponsor, and medical officer. Response will be determined by WHO criteria. MPNSTs are typically complex, non-spherical tumors and 2-D may thus reflect changes in tumor size better than 1-D (RECIST). In addition, previous and ongoing trials of refractory phase II MPNST trials (erlotinib and bevacizumab/everolimus) have used WHO criteria and will allow for direct comparisons. However, patients who experience disease progression based on WHO criteria, but who are receiving benefit from ganetespib and sirolimus as evidenced by decrease in the MPNST growth rate or clinical improvement may continue treatment with ganetespib and sirolimus provided they have:

- stable disease based on RECIST 1.1 criteria
- not met other off treatment / study criteria (Sections 5.8 and 5.9)

Treatment may continue until criteria for disease progression by RECIST 1.1 are met.

Secondary objectives will be to determine changes in pharmacodynamic parameters including phospho-S6, AKT phosphorylation, pEIF2α, Hsp70, and G6PD in tumor tissues from diagnosis/recurrence, and on treatment if feasible. These markers will also be evaluated in peripheral blood mononuclear cells at baseline and during treatment. Changes in patient reported pain and impact of pain on daily activities will also be assessed before and during treatment.

2.7 Correlative Studies Background

1) Pharmacokinetics

We will describe the plasma pharmacokinetic profile of ganetespib and sirolimus when administered in combination therapy. Pre-therapy levels will be drawn at baseline. Pharmacokinetic analysis will occur on cycle 1, day 15 as to capture steady state sirolimus levels. Ganetespib has previously demonstrated no accumulation. On day 15: Ganetespib will be collected at 0h, 0.5h, 50min* (to be drawn 10 minutes prior to the end of infusion), 2h, 4h, 6h, 8h, and 24 hours. Sirolimus will be collected at hours 0, 1, 2, 4, and 24 hours. All patients enrolled in the phase I portion will be required to participate in pharmacokinetic testing. In the phase II component, up to 10 patients will have optional pharmacokinetic analysis performed for broader experience at the MTD/recommended dose.

Drug PK	Pre-therapy levels	Day 15: Time							
Ganetespib	X	0h	0.5h	50min*	2h	4h	6h	8h	24h
Sirolimus	X	0h		1h	2h	4h			24h

There are no additional required sirolimus trough levels. However, sirolimus trough levels can be drawn if concerns for toxicity or any other clinical indication, and recommendations to be discussed with principal investigator.

*If infusion duration is prolonged, draw sample 10 minutes prior to the end of infusion.

2) Pharmacodynamics

Correlative studies evaluating pharmacodynamic parameters on Hsp inhibition, mTOR inhibition, UPR activation, and oxidative stress will be explored in tumor tissue and peripheral blood mononuclear cells. Hsp70 levels increase when Hsp90 is effectively inhibited and has been used as a pharmacodynamic response to Hsp90 inhibitors [73]. Phospho-S6 is the marker of choice in mTOR PD studies [59,74] and recent data indicate that baseline expression could have predictive value [75]. The limited clinical efficacy of mTOR inhibitors have been proposed to result from AKT activation that can occur via the negative feedback pathways [76]. Sirolimus did not induce AKT activation in MPNSTs *in vivo* [27], and combined rapamycin/IPI-504 did not suppress AKT phosphorylation or expression levels in the mouse model, indicating that this combination is not more effective because it inhibits AKT [30]. EIF2α phosphorylation is a marker of UPR activation [28] and was observed in the preclinical model as a marker of ER stress [30]. Hsp90 inhibition appears to stimulate ROS production, and sirolimus appears to enhance these effects by suppressing endogenous anti-oxidants [30]. Glucose 6-phosphate dehydrogenase (G6PD) has a well-established role in protecting cells from oxidative stress via its effects on glutathione production, one of the most important endogenous cellular antioxidants [77-79]. G6PD expression can be suppressed by mTOR inhibitors *in vitro* through inhibitory effects on the transcription factor SREBP1 [80]. Dr. Cichowski's lab demonstrated that sirolimus potently suppressed G6PD mRNA levels in MPNST tumor tissue [30]. Sirolimus and Hsp90 inhibition combined dramatically suppressed G6PD mRNA and protein expression MPNSTs *in vivo*.

We will explore changes in pharmacodynamic parameters: Hsp70, Phospho-S6, AKT phosphorylation, EIF2α, and G6PD in peripheral blood mononuclear cells performed on day 1 prior to ganetespib and sirolimus administration, and on day 15, 6 hours post drug administration. We will also obtain archival tissue at diagnosis and/or recurrence to evaluate these tests if available. For consenting patients with tumors accessible safely by percutaneous biopsy, on the phase II component only, we will obtain tumor biopsy at baseline and then on day 8 or 15 of cycle 1 at some time point within 12 hours after infusion of ganetespib.

3) Patient reported pain and impact of pain of daily activities

Pain associated with a mass was found to be greatest risk factor associated with the development of MPNSTs in NF1 [12]. Pain may also serve 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 3, 5, 9 and 13. We will explore the relationship in the 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 Brief Pain Inventory will be used to assess the impact of pain on daily activities. These scales have been placed on a single page to simplify administration (Appendix V). Total administration time is less than 3 minutes.

i) The Numerical Rating Scale-11 (NRS-11) is a self-report segmented 11-point numeric scale that assesses pain severity [81]. 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.

ii) The Brief Pain Inventory is a 7-item self-report questionnaire that measures the extent to which pain interferes with daily functioning [82]. Patients are asked to indicate how much pain interfered with various activities (general activity, mood, walking, normal work, relations with other people, sleep, and enjoyment of life) in the past week, with scores ranging from 0 (does not interfere) to 10 (completely interferes). A total score is obtained by taking the mean of the scores for all 7 items; thus, the total pain interference score can range from 0 to 7. This scale takes less than 2 minutes to complete.

4) Volumetric Tumor measurements

Using volumetric 3-dimensional MRI analysis of MPNSTs, we may be able to more sensitively monitor response to ganetespib and sirolimus compared to conventional two-dimensional MRI and one-dimensional MRI data analysis. Volumetric MRI analysis has become the standard method to evaluate the growth rate of plexiform neurofibromas on clinical trials [83,84]. This method may have utility for MPNSTs, which are typically large and have a complex shape.

Volumetric MRI analysis will be performed centrally at the NIH as a secondary objective and compared to standard response evaluation with 1D-and 2D- measurements. MRI studies performed for response evaluation will be used for volumetric analysis, and no MRI sequence other than STIR MRI (Short T1 inversion recovery), which is commonly used in the evaluation of sarcomas and does not require contrast administration, will be required.

3. PATIENT SELECTION

Eligibility Criteria

Patients must have baseline evaluations performed prior to the first dose of study drug and must meet all inclusion / exclusion criteria. Results of all baseline evaluations, which assure that all inclusion and 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 the 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.

3.1 Inclusion Criteria

3.1.1 Age ≥ 16 years

3.1.2 Phase I: Patients with unresectable, recurrent, or metastatic histologically confirmed soft tissue or bone sarcoma of one of the following subtypes:

- Leiomyosarcoma*
- Liposarcoma*
- MFH/pleomorphic undifferentiated sarcoma*
- Rhabdomyosarcoma*
- Malignant peripheral nerve sheath tumor (MPNST)*
- Osteosarcoma*
- Ewings*
- Alveolar soft part sarcoma
- Malignant giant cell tumor of bone
- Desmoplastic small round blue cell tumor
- Synovial Sarcoma
- Undifferentiated sarcoma

*Patients with leiomyosarcoma, liposarcoma, osteosarcoma, Ewings, MPNST, rhabdomyosarcoma or MFH must have documentation that they have received, not been eligible for, or refused at least one prior chemotherapy regimen prior to enrollment.

Phase II: Patients 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. 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.

3.1.3 For patients with NF1, diagnostic criteria leading to the diagnosis of NF1 and other NF1 findings must be documented on the eligibility checklist and on the form provided in Appendix IV.

Diagnostic criteria for NF1 are (NIH Consensus Conference 1987):
Presence of 2 or more of the following criteria:

1. Six or more café-au-lait spots (≥ 0.5 cm in prepubertal subjects or ≥ 1.5 cm in postpubertal subjects)
2. ≥ 2 neurofibromas or 1 plexiform neurofibroma
3. Freckling in the axilla or groin
4. Optic glioma
5. Two or more Lisch nodules

6. A distinctive bony lesion (dysplasia of the sphenoid bone or dysplasia or thinning of long bone cortex)
7. A first degree relative with NF1

3.1.4 ECOG Performance Status of ≤ 2.

3.1.5 Must be able to swallow whole pills.

3.1.6 Patients must have measurable disease, defined as at least one tumor that is measurable (defined as those that can be accurately measured in at least two dimensions (longest diameter ≥ 20 mm with conventional techniques or ≥ 10 mm using spiral CT scan) in two dimensions on CT or MRI scan. Baseline radiologic scans must be performed within 4 weeks of starting treatment.

3.1.7 Adequate organ function within 2 weeks of Day 1 of study defined as:

3.1.7.1 Adequate hematologic function as shown by: ANC ≥ 1.0 x 10^9/L, Platelets ≥ 75,000 x 10^9/L, Hgb > 9 g/dL (transfusion of packed red blood cells allowed).

3.1.7.2 Adequate liver function as shown by: serum bilirubin ≤ 1.5 x ULN, ALT and AST ≤ 3.0 x ULN (≤ 5x ULN in patients with liver metastases).

3.1.7.3 Fasting serum cholesterol ≤ 300 mg/dL OR ≤ 7.75 mmol/L AND fasting triglycerides ≤ 300 mg/dL OR < 3.42 mmol/L. NOTE: In the case one or both of these thresholds are exceeded, the patient can only be included after initiation of appropriate lipid lowering medication.

3.1.7.4 Serum creatinine ≤ ULN or creatinine clearance > 60 ml/min/1.73 m^2

3.1.7.5 Adequate Cardiac Function defined as:

- No history of congenital prolonged QTc syndrome, NYHA Class III or IV congestive heart failure (CHF)
- No clinically significant cardiac arrhythmias, stroke or myocardial infarction within 6 months prior to enrollment
- Second or third degree atrioventricular block unless treated with a permanent pacemaker
- Complete left bundle branch block
- QTcF ≤ 480 ms. Note: Patients with Grade 1 prolonged QTcF (450-480 msec) at the time of study enrollment should have correctable causes of prolonged QTc addressed if possible (i.e. electrolytes, medications).
- Use of medications that have been linked to the occurrence of torsades de pointes (see Appendix VI) within the last 7 days.

3.1.8 Prior therapy: Patients must have fully recovered from the acute toxic effects of

all prior anti-cancer therapy. Recovery is defined as a toxicity < grade 2 (CTCAE v 4.0), unless otherwise specified in the inclusion/exclusion criteria.

a. Myelosuppressive chemotherapy: Patients must have not received myelosuppressive chemotherapy within 3 weeks of enrollment onto this study.
b. Biologic agent: At least 7 days after the last dose of biologic agent. For agents that have known adverse events occurring beyond 7 days after administration, this period must be extended beyond the time which adverse events are known to occur.
c. Immunotherapy: At least 42 days after the completion of any type of immunotherapy, e.g. tumor vaccines.
d. Monoclonal antibodies: Patients may not have received monoclonal antibodies within 3 weeks of enrollment onto this study.
e. Radiation: At least 14 days after local palliative XRT (small port); or at least 4 weeks otherwise.
f. Stem Cell Transplant: No evidence of graft versus host disease, and ≥ 2 months must have elapsed since transplant.
g. Hematopoietic growth factors: At least 7 days must have elapsed since completion of therapy with a growth factor. At least 14 days must have elapsed after receiving pegfilgrastim.

3.1.9 Fertile men and women of childbearing potential must agree to use an effective method of birth control from Day 1 of study and for 120 days after last study drug administration in both sexes. Effective methods of birth control includes: surgically sterile, barrier device (condom, diaphragm), contraceptive coil, abstinence, or oral contraception.

3.1.10 Written, voluntary informed consent.

3.1.11 Durable power of attorney (DPA): All patients ≥ 18 years of age will be offered the opportunity to assign DPA so that another person can make decisions about their medical care if they become incapacitated or cognitively impaired.

3.2 Exclusion Criteria

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

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

3.2.3 Patients should not receive immunization with attenuated live vaccines within one week of study entry or during study period.

3.2.4 Uncontrolled brain or leptomeningeal metastases, including patients who continue to require glucocorticoids for brain or leptomeningeal metastases.

3.2.5 Other malignancies within the past 3 years except for adequately treated carcinoma of the cervix or basal or squamous cell carcinomas of the skin. Stable NF1 related tumors, such as optic pathway tumors, which do not require treatment at time of study enrollment, will not be considered an exclusion criterion.

3.2.6 Patients who have 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 O_2 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 tests are not required.

- Significant vascular disease (e.g. aortic aneurysm, symptomatic peripheral vascular disease) within 6 months prior to enrollment

- Uncontrolled diabetes as defined by fasting serum glucose >1.5 x ULN

- A known history of HIV seropositivity, as immune deficiency increases the risk for opportunistic infection.

- Impairment of gastrointestinal function or gastrointestinal disease that may significantly alter the absorption of sirolimus (e.g., ulcerative disease, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome or small bowel resection).

- Patients with an active, bleeding diathesis or significant coagulopathy (in absence of therapeutic anticoagulation).

3.2.7 Female patients who are pregnant or breast feeding, or adults of reproductive potential who are not using effective birth control methods. (Women of childbearing potential must have a negative urine or serum pregnancy test within 7 days prior to administration of study drugs)

3.2.8 Patients with a known hypersensitivity to rapamycins (sirolimus, temsirolimus, everolimus) or to its excipients. Excipients: Tablets: butylhyroxytoluene/butylated hydroxytoluene (BHT), magnesium stearate, lactose monohydrate, hypromellose/hydroxypropyl methylcellulose, crospovidone, lactose anhydrous. The excipients comply with the requirements of the applicable compendial monographs (Ph. Eur., USP/NF).

3.2.9 Patients unwilling or unable to comply with the protocol.

3.2.10 CYP3A4/CYP2C19 substrates: See the list on Appendix II: Patients should not have received these medications within 1 week of entry and is not allowed while on study.

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

3.2.12 Enzyme inducing anticonvulsants: Patients may not be taking enzyme –inducing anticonvulsants, and may not have received these medications within 1 week of entry, as these patients may experience different drug disposition. These medications include:

- Carbamazepine (Tegretol)
- Felbamate (Felbtol)
- Phenobarbitol
- Phenytoin (Dilantin)
- Primidone (Mysoline)
- Oxcarbazepine (Trileptal)

3.3 Inclusion of Women and Minorities

Subjects of both genders and from all racial and ethnic groups are eligible for this trial if they meet the criteria. To date, there is no information that suggests differences in absorption, metabolism, or 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, but in a phase I/II trial which will accrue a limited number of patients, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully

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 Table	
Days 1, 8, 15	Ganetespib IV over 1 hour
Days 1-28	Sirolimus once daily
Day 28	End of Cycle

Ganetespib will be provided by Synta pharmaceuticals. Ganetespib will be diluted to appropriate dose in D5W prior to administration. Ganetespib will be given at hour 0 on days 1, 8, and 15 intravenously over 1 hour. For patients who have infusion reactions or it is felt in the best interest of the patient to have the infusion given for longer duration, ganetespib may be administered over up to 2 hours and will not be considered a protocol deviation. The infusion duration should be noted on the medical administration record, case report form and pharmacokinetic worksheet, if applicable. *All efforts should be made to adhere to this schedule. However, to provide some flexibility to changes in patients' schedules and holidays for long-term treatment, +/- 1 day in changes will not be considered a protocol deviation and will not require reporting to the NCI, IRB, or FDA.

Required Pre-medications:

1) Loperamide 2 mg should be given 1 to 2 hours prior to ganetespib administration and then every 4 hours for 12 hours post infusion to all patients as prophylactic diarrhea management.

2) Dexamethasone 10 mg IV and diphenhydramine HCl 25 mg to 50 mg IV or PO (or therapeutic equivalents) should be given prior to administration of each ganetespib infusion. The premedication may be modified per institutional guidelines or as clinically indicated.

Based on timing of laboratory correlates (pharmacokinetics and pharmacodynamics), we would recommend that treatment day 1 to start on a Monday, Tuesday, or Wednesday if at all feasible.

Sirolimus will be purchased commercially (2 mg 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 given once daily continuously with no breaks in between cycles. On days when sirolimus and ganetespib are given together, they should begin at hour 0 together. Sirolimus should be taken at approximately the same time every day, preferably in the morning, consistently with or without food. If a patient misses a dose, the dose may be taken within 6 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. All doses prescribed and dispensed to the patient and all dose changes during the study will be recorded in the patient diary (Appendix III).

A cycle of therapy is considered to be 28 days.

Ganetespib drug doses should be adjusted based on BSA determined prior to the beginning of each cycle.

5.2 Criteria for starting subsequent cycles

A cycle may be repeated every 28 days if the patient has at least stable disease, has not experienced dose-limiting toxicity, and has recovered from the prior cycle as evidenced by return to baseline eligibility. Patients who experience progression based on WHO criteria as outlined in section 11.1.4, but in the opinion of the treating investigator are deriving benefit from treatment and do not meet off treatment or off study criteria (Sections 5.8 and 5.9), may continue on therapy as long as they have not met progression by RECIST 1.1 criteria (11.1.7). The study PI must be notified and SARC RECIST 1.1 form in Operations Manual must be sent to SARC demonstrating at least stable disease.

A cycle can be extended by an additional 14 days (to day 42) to allow for recovery without modifying the dose of the drug on subsequent cycles if toxicity did not meet dose limiting criteria. Patient must not met one of the off protocol therapy or off study criteria defined in section 5.8 and 5.9. A cycle may be administered up to 13 times. Patients who in the opinion of the treating investigator are deriving benefit may be eligible for additional cycles, but will be determined at the discretion of the study PI, sponsor, and medical officer.

5.3 Dose Escalation Schema [Phase I component]- Completed

5.3.1 Inter-patient Escalation

Dose Level	*Ganetespib (mg/m^2) IV days 1, 8, 15 every 28 days*	*Sirolimus (mg)*	
		Loading dose C1 D1 only	*Maintenance PO once daily continuous*

-2	*100*	*6*	*2*
-1	*150*	*6*	*2*
*1**	*150*	*12*	*4*
2	*200*	*12*	*4*
** Starting dose*			
Each cycle is considered 28 days			

5.3.2 Criteria for Dose Escalation

- *Cohorts of 3 to 6 patients will be treated with drug combination at each dose level. When a minimum of three patients who are evaluable for toxicity have completed one cycle of therapy at a dose level without evidence of dose-limiting toxicity (DLT) (section 5.3.3), subsequent patients may be enrolled at the next higher dose level.*
- *If DLT is observed in 1 patient from the initial cohort of 3 patients at a given dose level, an additional 3 patients will be entered at that dose level. If none of these additional patients experiences a DLT (1/6 with DLT), the dose will be escalated. If ≥ 1 of the additional patients experience a DLT (≥ 2/6 with DLT), the MTD has been exceeded, and the next lower dose level will be considered the MTD.*
- *If the MTD has been exceeded at the starting dose level, then the subsequent cohort of patients will be de-escalated per schema above.*

5.3.3 Definition of Dose Limiting Toxicity (DLT) (Criteria for entire study)

Toxicity will be graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website (http://ctep.cancer.gov). Any suspected or confirmed dose-limiting toxicity should be reported within 24 hours to the overall study Principal Investigator.

DLT will be defined as any of the following events that are possibly, probably, or definitely attributable to ganetespib or sirolimus. The DLT observation period for the purposes of dose escalation will be the first cycle of therapy. Dose limiting hematological and non-hematological toxicities are defined below:

5.3.3.1 Non-hematological DLT

- Any Grade ≥ 4 non-hematological toxicity
- Any Grade 3 non-hematological toxicity with the specific exclusion of
  - Grade 3 nausea and vomiting of < 3 day duration.
  - Grade 3 Diarrhea ≤ 3 days duration
  - Grade 3 ALT/AST that returns to meet initial eligibility criteria within 7 days of study drug interruption and that 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.

- Allergic reactions that necessitate discontinuation of study drug will not be considered dose-limiting.
- Grade 2 non-hematological toxicity that persists for ≥ 7 days and is considered sufficiently medically significant or intolerable by patients that it requires treatment interruption.
- Any adverse event requiring interruption of study drug for ≥ 7 days or which recurs upon drug challenge.

5.3.3.2 Hematological DLT

- Grade 4 Thrombocytopenia
- Grade 4 Neutropenia
- Grade 4 Anemia

5.3.4 Intra-Patient Escalation

Intra-patient dose escalation is not allowed.

5.3.5 Definition of Maximum Tolerated Dose (MTD)

The MTD is defined as the dose level immediately below the dose at which ≥ 33% of patients in a cohort experience a DLT.

- In order to escalate a dose level, < 33% of patients in a cohort should have a DLT.
- At least 3 patients in a cohort must be evaluable for the definition of the MTD in order to escalate.
- If 1 out of 3 patients in a cohort experience a DLT, then at least 6 patients must be enrolled and evaluable for the definition of MTD prior to dose escalation.

To determine extended tolerability, toxicities observed during the first treatment cycle will be used to define the MTD. A patient will be considered evaluable for definition of the MTD if at least 85% of the prescribed sirolimus dose has been administered to the patient during the first treatment cycle (unless held for toxicity) based on diary review and pill count of returned drug, and patient must have received all 3 doses of ganetespib in the cycle. If a discrepancy occurs, pill count will be used for adherence measurement. If a patient has less than 85% adherence for reasons other than toxicity, the patient will be replaced in the cohort. In addition, anyone who receives one or more doses and experiences a DLT will be considered evaluable for definition of MTD.

5.4 Dosing for Phase II component

Patients will be treated at the recommend dose from the Phase I component of this trial, which is dose level 2 with ganetespib 200 mg/m^2/dose IV given over one hour x 3 weeks

(days 1, 8, 15), 1 week off combined with sirolimus 4 mg by mouth once daily continuously (loading dose of 12 mg given on day 1 of cycle 1 only). One cycle equals 28 days.

5.5 General Concomitant Medication and Supportive Care Guidelines

Patients must be instructed not to take any additional medications (including over-the-counter products) during the trial without prior consultation with the investigator. All medications taken at the time of screening should be recorded. If concomitant therapy must be added or changed, the reason and name of the drug/therapy should be recorded.

In general, the use of any concomitant medication/therapies deemed necessary for the care of the patient are allowed, including drugs given prophylactically (e.g. antiemetics), with the following exceptions:

- No other investigational therapy should be given to patients
- No chronic treatment with systemic steroids or another immunosuppressive agent (for example, cyclosporine) with the exception of patients with endocrine deficiencies who are allowed to received physiologic or stress doses of steroids if necessary.
- The CYP3A4/ CYP2C19 substrates listed on Appendix II and enzyme inducing anticonvulsants listed in section 3.2.10 and 3.2.11, and 3.2.12 are prohibited on this trial. Exercise caution when using sirolimus with other drugs or agents that are modulators of CYP3A4.
- Use of medications that have been linked to the occurrence of torsades de pointes (See Appendix VI) are prohibited.
- Patients should not receive immunization with attenuated live vaccines during the study period. Close contact with those who have received attenuated live vaccines should be avoided. Examples of live vaccines include intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella and TY21a typhoid vaccines.

5.5.1 Concomitant Cancer and other Therapy

- Concurrent cancer therapy, including chemotherapy, radiation therapy, immunotherapy, or biologic therapy may NOT be administered.

5.5.2 Supportive Care

- Appropriate antibiotics, blood products, antiemetics (EXCEPT systemic steroids and aprepitant), fluids, electrolytes and general supportive care are to be used as necessary. Platelets should be transfused for thrombocytopenia following institutional guidelines. All blood products will be administered following institutional guidelines to prevent graft-versus-host disease. Corticosteroids are permissible as premedication for blood product transfusions, or as treatment for an acute allergic reaction.
- Diarrhea management (refer to Operations Manual):

- All patients will receive prophylactic loperamide 2 mg prior to and every 4 hours during the first 12 hours post infusion of ganetespib.
- For patients with Grade 1 or 2 Diarrhea: Loperamide as an initial 4 mg dose followed by 2 mg doses every 4 hours (Do not exceed 16 mg in 24 hours). Patient should continue until free of diarrhea for 12 hours.
- For Grade 3 or 4 Diarrhea: Consider hospitalization with IVF if clinically indicated and antibiotics as appropriate.

- **Patients must take some form of PCP prophylaxis while on sirolimus**. For example: trimethoprim/sulfamethoxazole as per institutional guidelines, or suitable alternative (Of note, injectable pentamidine is on the list of medication at risk for Torsades de Pointes [Appendix VI]. This does not apply to inhaled pentamidine. Inhaled pentamidine is a suitable alternative for PCP prophylaxis).
- Good oral hygiene and mouth care are encouraged, as mucositis is one of the toxicities of sirolimus.
- Growth Factors that support platelet or white cell number or function can only be administered for culture proven bacteremia, clinical sepsis, or invasive fungal infection with neutropenia. ASCO guidelines and regulatory authority labeling for providing growth factor support are recommended.
- Vaccinations: Patients receiving immunosuppressants, including sirolimus, should not be administered live vaccines. In addition, the response to vaccines (non-live) administered while the patient is immunosuppressed can be variable, and clinicians should check titers following for response if a non-live vaccine must be administered during this time.

5.5.3 Management for Surgery

Patients undergoing minor surgery should hold study drugs for 2 weeks prior to procedure, if feasible, and for 2 weeks after. Patients will be considered evaluable for toxicity and/or response as long as they meet criteria as defined in Section 11.1.1.

5.6 Duration of Therapy

Treatment may continue for up to 1 year (13 cycles) in the absence of disease progression or unacceptable adverse events. See section 5.2 for criteria to start subsequent cycles. Patients who experience progression based on WHO criteria as outlined in section 11.1.4, but in the opinion of the treating investigator are deriving benefit from treatment and do not meet off treatment or off study criteria (Sections 5.8 and 5.9), may continue on therapy up to 1 year as long as they have not met progression by RECIST 1.1 criteria (11.1.7). After 13 cycles, patients who in the opinion of the treating investigator are deriving benefit may be eligible for additional cycles, but will be determined at the discretion of the study PI, sponsor, and medical officer.

5.7 Duration of Follow Up

Patients will be followed until 30 days after the last dose of ganetespib and sirolimus or longer if the patient is removed from treatment with ganetespib and sirolimus for unacceptable adverse events. Adverse events will be followed until resolution or stabilization of the adverse event as detailed in Section 7.1.2.

5.8 Criteria for Removal from Study Treatment

1. Disease progression by WHO criteria. For patients who by their treating physician feel as though they are deriving benefit from study, may stay on study as long as they do not meet progression defined by RECIST 1.1 (Section 11.1.7).
2. Intercurrent illness that prevents further administration of treatment
3. Unacceptable adverse event(s), including significant irreversible grade 4 toxicity attributed to sirolimus or ganetespib
4. Patients with Grade 4 QTcF prolongation and torsades de pointes or polymorphic ventricular tachycardia or signs and symptoms of serious arrhythmia OR repeated grade 3 or higher QTcF prolongation must discontinue treatment with ganetespib
5. Greater than 3 weeks have elapsed since the last dose of sirolimus or greater than 8 weeks since the last dose of ganetespib
6. Patient decides to withdraw from the study
7. General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

5.9 Off Study Criteria

1. Thirty days after the last dose of investigational agent
2. Death
3. Lost to follow-up
4. Withdrawal of consent for any further data submission
5. Entry onto another therapeutic study

6. DOSING MODIFICATIONS/ MANAGEMENT FOR SPECIFIC SIDE EFFECTS

Dosing changes for ganetespib related toxicities are described in Section 6.3 and for sirolimus in Section 6.4. 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 ganetespib or sirolimus, the patient can continue on study receiving the agent, which is tolerated for as long as no other off treatment criteria are met. The study PIs should be contacted to discuss questions regarding toxicity attribution and to discuss patients who may meet criteria to continue treatment with either agent alone.

6.1 Dose modifications for non-hematological toxicity

1. If a patient experiences a non-hematological dose limiting toxicity as defined in Section 5.3.3.1, the attributable treatment will be withheld. If the toxicity resolves to

meet on study parameters within 14 days of drug discontinuation, the patient may resume treatment at a reduced dose. Doses reduced for toxicity will not be re-escalated, even if there is minimal to no toxicity with the reduced dose.

2. If toxicity does not resolve to meet on study entry parameters within 14 days of drug discontinuation, the patient must discontinue the protocol therapy.
3. If dose-limiting toxicity recurs in a patient who has resumed treatment at a reduced dose, the patient must discontinue the protocol therapy.
4. Patients with reported Grade 3 severity at any of the specified ECG time points (QTcF > 500 ms), ganetespib should be withheld. Ganetespib may be restarted at dose reduction (Section 6.3) when QTcF is ≤ Grade 1. While the QTcF prolongation is Grade 3 or higher, patients should have additional ECG monitoring until QTcF prolongation returns to ≤ Grade 1. Patients with grade 3 QTcF prolongation who restart at reduced dose, should have 24 hour +/- 2 hours EKG performed after reduced dose. If no evidence of QTcF prolongation, then patient will only require pre-dose EKG every other cycle as previously performed.

6.2 Dose modifications for hematological toxicity

1. If a patient experiences hematological dose-limiting toxicity as defined in Section 5.3.3.2, the attributable treatment will be withheld. Counts should be checked twice weekly during this time. When toxicity resolves to meet study parameters within 14 days of drug discontinuation, the patient may resume treatment at the reduced dose. Doses reduced for toxicity will not be re-escalated, even if there is minimal to no toxicity with the reduced dose.
2. If toxicity does not resolve to meet on study entry parameters within 14 days of drug discontinuation, the patient must discontinue protocol therapy.
3. If dose-limiting toxicity recurs in a patient who has resumed treatment at a reduced dose, the patient must discontinue the protocol therapy.

6.3 Ganetespib dose level modifications

A single dose reduction of 30% is allowed for toxicities outlined Section 5.3.3.

6.4 Sirolimus dose level modification

A single dose reduction of 50% is allowed for toxicities outlined in Section 5.3.3.

6.5 Management of Specific Side Effects

6.5.1 Management of hypersensitivity reactions to ganetespib infusions

Ganetespib contains a surfactant (polysorbate 80) that has been associated with hypersensitivity reactions in other medications administered by infusion. Symptoms have included pruritis, flushing, shortness of breath, chest tightness, dizziness, headache, increased systolic BP and HR. Therefore, we are requiring premedication with steroid and anti-histamine prior to each ganetespib infusion (see Section 5.1). Despite premedication, if an infusion hypersensitivity reaction

to ganetespib is suspected, the following management is provided as guidance. Treatment should be based on clinical presentation. Institution specific procedures and regimens may be appropriate in lieu of these guidelines.

Mild or moderate symptoms:

- Stop ganetespib infusion
- Administer IV dexamethasone and diphenhydramine HCl or therapeutic equivalent
- After recovery from symptoms, resume ganetespib infusion at reduced rate
- In subsequent cycle, consider optimizing premedication regimen (e.g., begin steroids the day before infusion or increase dose of steroids)

Severe Symptoms (such as hypotension requiring pressor therapy or IV fluids, angioedema, respiratory distress requiring bronchodilator therapy, or generalized uticaria):

- Stop ganetespib infusion
- Administer IV dexamethasone, diphenhydramine HCl or therapeutic equivalent
- Add adrenaline (1:1000) or bronchodilators as indicated

In subsequent cycles, optimize the premedication regimen (e.g., begin steroids the day before infusion or increase the dose of steroids) and reduce the flow rate of the ganetespib infusion.

The following is an example of infusion premedication regimen:

- Dexamethasone 12 mg orally and diphenhydramine HCl 25-50 mg orally approximately 12 to 24 hours prior to next dose of study
- Repeat dexamethasone 12 mg orally and diphenhydramine HCl 25-50 mg orally approximately 4-6 hours prior to the re-challenge.

If severe symptoms recur with optimal premedication, treatment with ganetespib must be discontinued.

6.5.2 Management of stomatitis/oral mucositis/mouth ulcers

Stomatitis/oral mucositis/mouth ulcers due to sirolimus should be treated using local supportive care. Please note that investigators in earlier trials have described the oral toxicities associated with sirolimus as mouth ulcers, rather than mucositis or stomatitis. If your examination reveals mouth ulcers rather than a more general inflammation of the mouth, please classify the adverse event as such. Please follow the paradigm below for treatment of stomatitis/oral mucositis/mouth ulcers:

1. For mild toxicity (Grade 1), use conservative measures such as non-alcoholic mouth wash or salt water (0.9%) mouth wash several times a day until resolution.

2. For more severe toxicity (Grade 2 in which case patients have pain but are able to maintain adequate oral alimentation, or Grade 3 in which case patients cannot maintain adequate oral alimentation), the suggested treatments are topical analgesic mouth treatments (i.e., local anesthetics such as benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol, or phenol) with or without topical corticosteroids, such as triamcinolone oral paste 0.1% (Kenalog in Orabase®). For intolerable grade ≥ 2 stomatitis/oral mucositis/mouth ulcers, sirolimus will be held, and restarted with a dose reduction by 1 dose level after recovery from toxicity to grade 1 or less.
3. Agents containing hydrogen peroxide, iodine, and thyme derivatives may tend to worsen mouth ulcers. It is preferable to avoid these agents.
4. Antifungal agents must be avoided unless a fungal infection is diagnosed. In particular, systemic imidazole antifungal agents (ketoconazole, fluconazole, itraconazole, etc.) should be avoided in all patients due to their strong inhibition of sirolimus metabolism, thereby leading to higher sirolimus exposures. Therefore, topical antifungal agents are preferred if an infection is diagnosed. Similarly, antiviral agents such as acyclovir should be avoided unless a viral infection is diagnosed.

Note: Stomatitis/oral mucositis should be appropriately graded using the functional grading given on the NCI-CTC for adverse events, version 4.

6.5.3 Management of hyperlipidemia and hyperglycemia

Treatment of hyperlipidemia should take into account the pre-treatment status and dietary habits. Blood tests to monitor hyperlipidemia must be taken in the fasting state. Grade 2 or greater hypercholesterolemia (> 300 mg/dL or 7.75 mmol/L) or Grade 2 or greater hypertriglyceridemia (> 300 mg/dL or > 3.42 mmol/L) 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. Note: Concomitant therapy with fibrates and an HMG-CoA reductase inhibitor is associated with an increased risk of a rare but serious skeletal muscle toxicity manifested by rhabdomyolysis, markedly elevated creatinine kinase (CPK) levels and myoglobinuria, acute renal failure and sometimes death. The risk versus benefit of using this therapy should be determined for individual patients based on their risk of cardiovascular complications of hyperlipidemia. Grade 4 **hypercholesterolemia** (> 500 mg/dL or > 12.92 mmol/L) hold sirolimus until cholesterol is < Grade 4. If triglycerides > 500-1,000, and HDL is low, consider fibrate or niacin. For triglycerides > 1000 mg/dL or >11.4mmol/L, hold sirolimus while instituting fibrate or niacin therapy until triglycerides are < Grade 4.

Grade 3 **hyperglycemia** has been observed in patients receiving sirolimus

therapy. In many cases the affected patients had an abnormal fasting glucose at baseline. Based on this finding, it is suggested that optimal glucose control should be achieved before starting a patient on sirolimus and should be monitored during sirolimus therapy.

6.5.4 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 in patients taking sirolimus. Some of these have been severe and on rare occasions, a fatal outcome was observed.

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 and 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.

Patients who develop radiological changes suggestive of non-infectious pneumonitis and have few or no symptoms may continue sirolimus therapy without dose alteration. Dose modifications and retreatment are described in Table 3 below.

Table 3: Management of non-infectious pneumonitis

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	Administer 100% of sirolimus 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.**

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Adverse Event and Laboratory Abnormalities

7.1.1 Clinical AEs

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 Common Terminology Criteria for Adverse Events v 4.0 (CTCAE) 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.

7.1.1.5 Definition of Serious Adverse Events

A serious adverse event (SAE) is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any Adverse Event that at any dose fulfils 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);
- requires 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.

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 WHO 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.

7.1.2 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

Grade 2 AEs judged to be clinically significant: Follow as clinically indicated.

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

7.1.3 Laboratory Test Abnormalities

Any laboratory result abnormality fulfilling the criteria for a serious adverse event 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)

7.1.4 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 eCRF.

7.2 Handling of Safety Parameters

7.2.1 Reporting of Adverse Events

All adverse events ≥ 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 SARC **within *24 hours*** of the site PI 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. SARC will report all serious adverse events to the Principal Investigator, Medical Monitor, Dr. Scott Okuno and to Synta within 1 working day.

SAEs must be reported on the MedWatch 3500A form included in the Operations Manual with the completed Fax/Email Coversheet and faxed/emailed to SARC (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 must report all pregnancies within 24 hours to the sponsor.

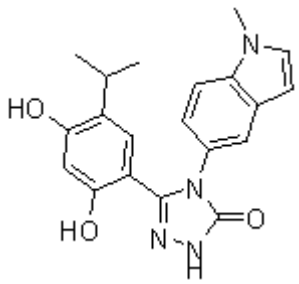
The investigator should counsel the patient; discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Depending on maternal/fetal outcomes, follow-up beyond pregnancy may be required.

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.

8. PHARMACEUTICAL INFORMATION

8.1 Ganetespib

Ganetespib, chemical name: 5-[2,4-dihydroxy-5-(1-methylethyl)phenyl]-2,4-dihydro-4-(1-methyl-1*H*-indol-5-yl)-3*H*-1,2,4-triazole-3-one, is a novel triazolone heterocyclic compound. Its molecular formula is $C_{20}H_{20}N_4O_3$. Ganetespib is a white to off-white powder with a molecular weight of 364.40 g/mol.



8.1.1 Preparation and Administration

The current ganetespib investigational product is a concentrate for solution for infusion provided in a single-use vial containing 300 mg /vial of ganetespib, as described in the Pharmacy Manual. The concentration of ganetespib is 25 mg/mL in a polyethylene glycol 300 (PEG 300), polysorbate 80 (Tween-80) and dehydrated alcohol non-aqueous solvent system. The drug product is a clear, colorless-to-pale-yellow solution, essentially free of visible particles. **Note: Ensure correct administration instructions are followed prior to use of either product (see Pharmacy Manual).**

Ganetespib Drug Product, 25 mg/mL, 300 mg/vial (identified with a dark blue color cap and applicable product label): Each vial contains 12 mL of deliverable volume (12.84 mL total including an overage per USP requirements) equivalent to 300 mg of ganetespib at a concentration of 25 mg/mL in a PEG 300, polysorbate 80, and dehydrated alcohol non-aqueous solvent system. The drug product, as noted, is a clear, colorless-to-pale-yellow solution.

The amount of ganetespib administered will depend upon the patient's body surface area. The drug product is diluted before infusion.

Ganetespib must be diluted prior to administration. **The appropriate drug administration instructions per the preparation guidelines must be carefully followed prior to use.** Refer to the Pharmacy Manual for detailed ganetespib preparation guidelines.

Based on preclinical data, use of vascular access devices (VADs) (such as ports and peripherally-inserted central catheters [PICCS]) containing silicone catheters for ganetespib administration is permitted. Use of VADs made of any other material is not permitted. Following ganetespib administration through a VAD, care should be taken to flush the line after each dose of study drug.

8.1.2 Formulation, Packaging and Labeling

Container/Closure

The ganetespib drug product is provided in a 30 mL type I amber glass vial fitted with a 20 millimeter stopper and sealed with an aluminum crimp and flip-off cap.

Starting with the 300 mg drug product, the storage condition will be changed to 20-25°C (68°F to 77°F) with excursions allowed between 15°C and 30°C (59°F and 86°F).

8.1.3 Agent Ordering

For this trial, Ganetespib will be provided by Synta Pharmaceuticals. Details will be provided in the Operations Manual.

8.1.4 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 (Section 8.7).

8.1.5 Destruction of ganetespib

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 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)

8.1.6 Ganetespib toxicities (please refer to most recent IB)

Single-Agent Studies

The following adverse reactions were observed in patients with cancer (either solid tumor or hematologic malignancies) receiving single-agent ganetespib at varying dose levels and schedules. Frequency (%) provided is based on the largest aggregate data set (n=402).

Very Common Adverse Reactions (≥ 10%)

The most common adverse reactions in 402 single-agent patients include diarrhea (80%), fatigue (53%), nausea (44%), decreased appetite (31%), vomiting (27%).

Table 1. Very Common (≥ 10%) Adverse Reactions, Single Agent Studies (n=402)

Preferred Term	**AEs (≥ 10%) (N=402) n (%)**
Diarrhea	320(79.6))
Fatigue	214 (53.2)
Nausea	177 (44.0)
Decreased appetite	126 (31.3)
Vomiting	109 (27.1)
Anemia	85(21.1)
Constipation	88 (21.1)
Insomnia	85 (8.0)
Abdominal pain	81 (20.1)
Headache	81 (20.1)
Dyspnea	70(17.4)
Back pain	63(15.7)
Blood alkaline phosphatase increased	65(16.2)
Aspartate aminotransferase increased	65(16.2)
Dehydration	59(14.7)
Weight decreased	62(15.4)
Hypokalaemia	55(13.7)

Alanine aminotransferase increased	62 (15.4)
Cough	52 (12.9)
Hyponatremia	54 (13.4)
Dizziness	53 (13.2)
Edema peripheral	53 (13.2)

Common (≥ 1% and < 10%) Adverse Reactions
Adverse reactions that were common, Grade ≥ 3, and related are increased lipase (3%), hypophosphatemia (2%), and lymphopenia (1%). One of the 10 patients with increased lipase had an event that was assessed as serious. None of the other common, Grade ≥ 3, and related AEs was assessed as serious

Table 2. Common (≥ 1% and < 10%); Grade ≥ 3 and Related and Serious Adverse Reactions in Single Agent studies (n=402)

Preferred Term[1]	**Grade ≥ 3 and Related AE**	**SAE**
Lipase increased	**10 (2.5)**	**1 (< 1)**
Hypophosphataemia	**8 (2.0)**	**0**
Lymphopenia	**5 (1.2)**	**0**
Asthenia	**5 (1.2)**	**1 (<1)**

[1] A patient counts once for a preferred term with any incidence of the event.
Note: This table includes only adverse reactions that are not listed in Table 1.

Adverse events that were uncommon (< 1%), related and serious are presented in Table 3.

Table 3. Uncommon (< 1%) Related, and Serious Adverse Reactions in Single-Agent Studies (N=402)

Amylase increase	**ECG QT prolonged**	**Infection**
Atrial fibrillation	**Failure to thrive**	**Infusion related reaction**
Cardiac arrest	**Febrile neutropenia**	**Pneumonia**
Colonic fistula	**Gastrointestinal Perforation**	**Rales**
Confusional state	**Hyperbilirubinaemia**	**Renal failure**
Pain in extremity	**Hypoxia**	**Syncope**
Blood lactate dehydrogenase increase	**Hyperuricemia**	**Thrombocytopenia**

Note: Related AEs are those with a Relationship of Possible, Probable, Definite, Unknown, or Missing
This table includes only adverse reactions that are not listed in Table 1 or Table 2.

Note: Recent review of database for ganetespib (September 2015, n=1509) has identified Gastrointestinal perforation (GIP) as potential rare risk (0.33%)

8.2 Sirolimus

Sirolimus is a macrocyclic lactone produced by Streptomyces hygroscopicus. 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

8.2.1 Preparation and Administration

For this trial 2 mg tablets will 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.

8.2.3 Agent Ordering

Sirolimus is commercially available. For this study, sirolimus will be supplied through the study. For agent ordering, see operations manual.

8.2.4 Agent Accountability

See Section 8.1.4

8.2.5 Sirolimus toxicities

Phase III studies of sirolimus in cyclosporine-based regimens revealed mild dose related thrombocytopenia and increases in serum triglycerides and cholesterol (in 40% to 50% of patients), which have responded well to HMG-CoA reductase inhibitors as well as fibricacid derivatives. Additionally, the unexpected finding of nephrotoxicity has been encountered but it is not clear whether it is directly attributable to sirolimus or to

apotentiation of cyclosporine's nephrotoxicity. Other common side effects includeleukopenia (20%), hypertension (45%), anemia (30%), headaches (29%), tremors (26%), nausea and vomiting (30%), diarrhea (34%), constipation (32%), urinary tract infections (25%), decreased phosphate (19%), decreased potassium (26%), peripheral edema (25%),rash and acne. The 5 mg dose did produce more marrow suppression and hyperlipidemia.

Side effects were related to drug concentration and were improved with maintenance of the sirolimus level between 10 to 20 ng/mL. Sirolimus's effect on the developing fetus is not known and is not recommended for administration to nursing mothers. Patients receiving immunosupressants, including sirolimus, should not be administered live vaccines. In addition, the response to vaccines (non-live) administered while the patient isimmunosuppressed can be variable and some clinicians should check titers following for response, if a killed vaccine must be administered while taking sirolimus. Recently, case reports have described a drug-induced pneumonitis in some patients receiving sirolimus, which was reversible upon discontinuation of the drug. mTOR inhibitors, such as sirolimus, have been shown *in vitro* to inhibit production of certain growth factors that may affect angiogenesis, fibroblast proliferation, and vascular permeability. There have been reports of impaired or delayed wound healing in patients receiving sirolimus including lymphocele and wound dehiscence. Patients with a body mass index (BMI) greater than 30 kg/m^2 may be at increased risk of abnormal wound healing based on data from the medical literature. There have also been reports of fluid accumulation, including peripheral edema, lymphedema, pleural effusion and pericardial effusions (including hemodynamically significant effusions and tamponade requiring intervention in children and adults), in patients receiving sirolimus. There is also new evidence of incisional hernia noted in patients who undergo transplant surgery. Hepatotoxicity has been reported, including fatal hepatic necrosis with elevated sirolimus trough concentrations. There have been reports of neutropenia, proteinuria, nephritic syndrome, pancytopenia, joint disorders, and lymphedema. Azoospermia has been reported with the use of sirolimus and has been reversible upon discontinuation of sirolimus in most cases. Hypersensitivity reactions, including anaphylactic/anaphylactoid reactions, angioedema, exfoliative dermatitis, and hypersensitivity vasculitis, have been associated with the administration of sirolimus. In addition, a 5-fold increase in the reports of tuberculosis among sirolimus (11/551) and comparator (1/273) treatment group was observed with 2:1 randomization scheme.

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

*From rxlist.com

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

9. CORRELATIVE/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.

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 Pharmacokinetics

Pharmacokinetic samples will be collected in all phase I patients. A pre-treatment sample will be obtained on day 1 prior to either medication. Pharmacokinetic samples for ganetespib will be performed by Synta pharmaceuticals. At baseline, pre-treatment levels will be drawn. On cycle 1, day 15, ganetespib pharmacokinetic samples will be collected at 0h, 0.5h, 50min.* (to be drawn 10 minutes prior to the end of infusion), 2h, 4h, 6h, 8h, and 24 hours post infusion. Pharmacokinetic analysis of samples for sirolimus will be performed by Cincinnati Medical Center in the laboratory of Kenneth Setchell. At baseline, pre-treatment levels will be drawn. On cycle 1, day 15, sirolimus pharmacokinetic samples will be collected at hour 0, 1, 2, 4, and 24 hours post dose. Collection instructions, PK sheets, handling, and shipment are provided in the operations manual.

*If infusion duration is prolonged, draw sample 10 minutes prior to the end of infusion.

9.1.1.2 Pain (Appendix V)

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 Brief Pain Inventory will be used to assess the impact of pain on daily activities. These scales have been placed on a single page to simplify administration. Total administration time is less than 3 minutes. These tests will be given prior to treatment and then prior cycle 3, 5, 9 and 13. Details regarding collection and assessment will be provided in the operations manual.

9.1.1.3 Volumetric MRI analysis

To evaluate the utility of 3-dimensional MRI analysis for MPNST, MRIs obtained for disease evaluation at baseline and as part of response evaluation will be analyzed using 3D MRI in addition to 1D and 2D analysis. This will be done centrally at NIH. Details regarding handling and shipping of MRI studies are provided in the Operations Manual.

9.1.2 Optional

9.1.2.1 Pharmacokinetics

Pharmacokinetic samples will be collected in up to 10 patients in the phase II component for data and experience at the recommended dose. Collection time will be as described above in Section 9.1.1.1.

9.1.2.2 Pharmacodynamics

Correlative studies evaluating pharmacodynamic parameters on Hsp inhibition (Hsp70), mTOR inhibition (phospho-S6 and Akt Phosphorylation), UPR activation (EIF2α phosphorylation), and oxidative stress (G6PD) will be explored in tumor tissue and peripheral blood mononuclear cells. 15 mL of blood will be collected prior to treatment with either agent and then on day 15 approximately 6 hours post ganetespib administration.

Diagnostic and/or relapsed archival MPNST tissue will be collected if available and feasible.

For patients on the phase II component only who consent to optional tumor biopsies and whose biopsies are easily accessible with percutaneous biopsy, tumor biopsy will be collected pre-treatment and then during cycle 1 on either day 8 or 15, within 12 hours after ganetespib administration to look at similar markers.

Detailed timing, instructions, handling, and shipment are provided in the operations manual.

10. STUDY EVALUATIONS AND STUDY CALENDAR

10.1 SCREENING STUDIES

The following procedures will be performed during the screening period within 2 weeks prior to treatment unless otherwise specified:

- Informed consent (within 1 month)
- History/demographics
- Physical exam
- Vital signs:
  Height (first visit), pulse, blood pressure, respiration rate, oxygen saturation by pulse oximeter, temperature, weight, and BSA.
- Performance Status (see Appendix I)
- Documentation of clinical findings of NF1 (Appendix IV)
- Hematology: CBC and differential, PT, PTT, INR.

- Serum Chemistry:
  Must include sodium, potassium, chloride, bicarbonate, calcium, phosphorous, magnesium, **FASTING** glucose, creatinine, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, uric acid, 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 to all females of childbearing potential within 7 days prior to starting treatment.
- EKG and QTc (QTc will be calculated as QTcF using the Fridericia's formula)
- Disease evaluation using appropriate test (CT and/or MRI) must be performed within 4 weeks prior to treatment

Patients should start study treatment within 2 weeks of enrollment/registration in the study database.

10.2 ON STUDY EVALUATIONS

The following procedures will be performed during the treatment period (Test required prior to subsequent cycles should be performed within a time frame of 4 days prior to cycle):

- History and physical: Weekly during cycle 1 and then prior to each cycle
- Vital Signs: Weekly during cycle 1 and then prior to each cycle: Pulse, blood pressure, respiration rate, oxygen saturation by pulse oximeter, temperature, weight, and BSA.
- Performance Status: Prior to each cycle
- Hematology: CBC weekly during cycle 1 and then every two weeks. If hematological DLT follow Section 6.2.
- Serum Chemistry: weekly during cycle 1 and then every two weeks. Must include sodium, potassium, chloride, bicarbonate, calcium, phosphorous, magnesium, glucose, creatinine, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase weekly during cycle 1, and then prior to every cycle.
- **FASTING** glucose and serum lipid profile (triglycerides, total cholesterol, HDL and LDL) prior to each cycle
- EKG and QTcF: During Cycle 1: 24 hours (+/- 2 hours) post infusion on Day 1 and then prior to every other cycle. If Grade ≥ 3, follow guidelines in Section 6.1.
- Disease evaluation using appropriate test (CT and/or MRI): Prior to cycles 3, 5, 7, 9, etc. Tumor imaging should NOT be delayed, if possible, if a subject temporarily or permanently suspends study drug treatment for toxicity or non-compliance with administration of drug. In patients who experience a PR or CR confirmation of the response after 4 weeks should be performed, if feasible.

- Administration of study drug:
  - A sirolimus diary will be kept by the patient and/or proxy to document each dose of drug taken. Toxicities experienced will be documented on the diary. The diary will be reviewed weekly during cycle 1and then prior to each cycle. Pill count will occur after each cycle.

Correlative studies (See Operations Manual for exact timing, collection, handling, shipping)

- Pharmacokinetics: Performed prior to treatment, and on Day 15, Cycle 1.
- Pharmacodynamics: Performed prior to treatment, and on Day 15, Cycle 1 (If patients consent to tumor biopsy, then can occur on day 8 or 15).
- Patient reported Pain and Impact Measurements: Performed prior to treatment and prior to cycles 3, 5, 9, and 13.
- MRI studies for volumetric analysis:
  MRI studies performed for restaging purposes at the time points of response evaluations will be analyzed using volumetric MRI analysis centrally in addition to standard 2-dimensional response evaluations (see Operations Manual).

10.3 OFF STUDY EVALUATIONS

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

- History and Physical exam
- Vital signs:
  Pulse, blood pressure, respiration rate, oxygen saturation by pulse oximeter, temperature and weight.
- Performance Status (see Appendix I)
- Hematology:
  Hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential, PT, PTT.
- Serum Chemistry:
  Must include sodium, potassium, chloride, bicarbonate, calcium, phosphorous, **FASTING** glucose, creatinine, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, uric acid, and **FASTING** serum lipid profile (triglycerides, total cholesterol, HDL and LDL). *FASTING = 12 hours prior to glucose and lipid panel
- Disease evaluation using appropriate test (CT and/or MRI).

10.4 STUDY EVALUATIONS

Studies to be obtained	Pre-Study	During Cycle 1	Prior to subsequent Cycles*	During Subsequent Cycles	End of Therapy
Informed Consent	X				
History and Physical with vitals [a]	X	Weekly	X		X
Performance Status [b]	X		X		X
Documentation of NF1 findings[c]	X				
CBC with differential	X	Weekly[d]	X	Every Two Weeks[d]	X
PT, PTT, INR	X				X
Serum Chemistry[e]	X	Weekly	X	Every Two Weeks	X
Fasting glucose and serum lipid profile[f]	X		X		X
Pregnancy Test[g]	X		Prior to odd Cycles (3, 5, 7, etc)		
EKG and QTcF[h]	X	Day 2 [i]	Prior to odd Cycles (3, 5, 7, etc)		X
Tumor Evaluation[J]	X		Prior to odd cycles (3, 5, 7, etc)		X
Pharmacokinetics[k]	X	X			
Pharmacodynamic Studies[l]	X	X			
Patient reported pain[m]	X		Prior to cycles 3, 5, 9, 13		
MRI for 3-D analysis[n]	X		Prior to odd cycles (3, 5, 7, etc)		X
Patient Diary[o]		Weekly	X	Prior to each cycle	X

[a] Vitals signs: Height (first visit only), pulse, blood pressure, respiratory rate, oxygen saturation by pulse oximeter, temperature, weight, and BSA calculation
[b] See Appendix I for performance status criteria
[c] See Appendix IV for documentation of findings for patients with NF1
[d] If a patient experiences a Grade 4 hematologic DLT, then CBCs should be checked twice weekly until recovery to Grade 3.
[e] Sodium, potassium, chloride, bicarbonate, calcium, phosphorus, magnesium, creatinine, glucose, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, uric acid
[f] Fasting= 12 hours prior to testing. Glucose and serum lipid panel (trigylcerides, total cholesterol, HDL, and LDL)
[g] Standard pregnancy test given to all females of childbearing age ≤ 7 days prior to starting medication, and prior to each radiological evaluation
[h] QTc calculated using Fridericia's formula
[i] To be done 24 +/- 2 hours hours post infusion of day 1 ganetespib
[j] Performed ≤ 4 weeks prior to trial entry. Disease evaluation using appropriate tests (CT/MRI) for volumetric analysis must be performed within 4 weeks of trial entry and prior to cycles 3, 5, 7, etc.
[k] See Section 2.6 and Operations manual for timing/instructions of PK collection
[l] See Section 2.6 and Operations manual for timing/instructions of PD collection
[m] See Section 2.6 and Operations for timing /instructions of Pain reported pain and impact on daily activities
[n] Only for MPNST patients
[o] See Appendix III.
* Can be performed within 4 days of prior to cycle

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

11.1.1 Definitions

Evaluable for toxicity for Phase I component: A patient will be considered evaluable for definition of the MTD if at least 70% of the prescribed sirolimus dose has been administered to the patient during the first treatment cycle based on diary review and pill count of returned drug [if a discrepancy occurs, pill count will be used for adherence measurement] and all 3 doses of ganetespib in the first cycle. If a patient has less than 70% adherence, the patient will be replaced in the cohort. In addition, anyone who receives one or more doses and experiences a DLT will be considered evaluable for definition of MTD.

In the phase II component: Patients who have received at least one dose of study drug will be considered evaluable for toxicity from the time of their first dose of ganetespib and sirolimus until the last evaluation on trial.

Evaluable for objective response:

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least two dimensions (longest diameter $\geq$ 20mm with conventional techniques or $\geq$10 mm using spiral CT scan). All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter < 20mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

Any patient who is enrolled and receives at least one dose of sirolimus and ganetespib will be considered evaluable for response provided: (1) the patient demonstrates progressive disease or death while on protocol therapy; (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. Patients who electively terminate therapy before receiving all ganetespib doses and $\geq$ 80% of the required sirolimus doses during the first treatment cycle and do not expire within 28 days from start of treatment will be replaced.

11.1.2 - Disease Parameters

Measurable, bidimensional: Malignant disease measurable in two dimensions by ruler with surface area determined by multiplying the longest diameter by the greatest perpendicular diameter.

Index Lesions: Index lesions should be selected on the basis of their measurability in two dimensions and their suitability for accurate repeated measurements (by imaging techniques CT or MRI). A sum of the product(s) of the longest diameter (LD) and greatest perpendicular diameter of all index lesions will be calculated and reported as the baseline sum. The baseline sum will be used as a reference by which to characterize the objective tumor response.

Non-index Lesions: All other lesions (or sites of disease) including any measurable lesions over and above the index lesions should be identified as non-index lesions and should also be recorded at baseline. Measurement of these lesions is not required, but the presence or absence 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 using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

Conventional CT and MRI: These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities may require specific protocols.

11.1.4 WHO response criteria

WHO will be used for this trial for several reasons:

1) MPNSTs are typically complex non spherical tumors, and 2-dimensional measurements may thus better reflect changes in tumor size than 1-dimensional measurements (RECIST).

2) The phase 2 trial of erlotinib and everolimus/bevacizumab, which will be used as a historical control for determination of time to progression, used WHO criteria. In order to

allow for the closest comparison, this trial will therefore use WHO response criteria.

11.1.4.1 Evaluation of Index Lesions

Complete Response (CR): Disappearance of all known disease, determined by two consecutive observations not less than 4 weeks apart.

Partial Response (PR): A ≥ 50% decrease in the total tumor load of the lesions that have been measured to determine the effect of therapy not less than four weeks apart. The observations must be consecutive.

> *Bidimensionally measurable:* single lesion, ≥ 50% decrease in tumor area (multiplication of longest diameter by the greatest perpendicular diameter); multiple lesions, a 50% decrease in the sum of the products of the perpendicular diameters of the multiple lesions.
>
> In addition there can be no appearance of new lesions or progression of any lesion.

Stable Disease (SD): A 50% decrease in total tumor area cannot be established nor has a 25% increase in the size of one or more measurable lesions been demonstrated.

Progressive Disease (PD): A ≥ 25% increase in the area of one or more measurable lesions or the appearance of new lesions.

11.1.4.2 Evaluation of Non-Index Lesions

Complete Response (CR): Complete disappearance of all known disease for at least four weeks.

Partial Response (PR): Estimated decrease in tumor area of ≥ 50% for at least four weeks.

Stable Disease (SD): No significant change for at least four weeks. This includes stable disease, estimated decrease of < 50%, and lesions with estimated increase of < 25%.

Progressive Disease (PD): Appearance of any new lesions not previously identified or an estimated increase of ≥ 25% in existent lesions.

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

Evaluation of Best Overall Response

Index Lesions	Non-Index Lesions	New Lesions	Overall Response	Best Response for this Category also requires:
CR	CR	No	CR	≥ 4 weeks confirmation
CR	PR/SD	No	PR	
PR	CR/PR/SD	No	PR	
SD	CR/PR/SD	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-index 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 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of objective progression or death.

11.1.7 RECIST 1.1 Criteria [85]

For patients who experience progression by WHO criteria as outlined above, but in the opinion of the treating investigator are deriving benefit from therapy and have not otherwise met off treatment or off study criteria, may continue on treatment as long as patient has not met progression by RECIST1.1 criteria outlined below.

Key points to RECIST version 1.1 are that 5 target lesions are identified and that changes in the largest diameter (unidimensional measurement) of the tumor lesions

are used in the RECIST v1.1 criteria. The sum of the longest diameter (LD) for all target lesions will be calculated and reported as the disease measurement.

Progression by RECIST 1.1 is at least 20% increase in the disease measurement, taking as reference the smallest disease measurement recorded since the start of treatment or the appearance of one or more new lesions or evidence of laboratory or clinical progression.

12. DATA REPORTING / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

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

12.1.2 Data Safety Monitoring and Medical Monitor

SARC is responsible for the Data Safety 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, Dr. Scott Okuno, 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 Operating 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.

Dr. Okuno 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 possibly or definitely related to participation and reports of events resulting in death must be promptly forwarded to Synta Pharmaceuticals. SARC will be responsible for forwarding reports to the SARC medical officer.

In addition to the Medical Monitor role, Scott Okuno, MD will function as the Department of Defense required "Independent Research Monitor". The 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 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 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 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 Independent Research Monitor is responsible for promptly reporting their observations and findings to the IRB.

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 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.

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.

12.1.3 Patient Accrual and Participating Centers

The study would be conducted through the SARC consortium in collaboration of the NF Consortium with the phase I component conducted at 5 centers with phase I expertise and the phase II at 10 consortium centers. We anticipate that accrual will take approximately 2.5 years.

This trial is posted at ClinicalTrials.gov website.

12.2 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 7.0) 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: SARC023@sarctrials.org

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).

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: SARC023@sarctrials.org

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 (see Section 4.2).

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.

MRI studies will be sent on CD or optical disk with patient identifiers to the NCI POB. CDs and optical disks will be locked in a filing cabinet with access only to authorized personnel. Imaging studies will be analyzed on 2 Sun Workstations, which are password protected, and limit access to authorized personnel.

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.

Adverse Events reporting will be performed as outlined in Section 7.0.

12.3 Data and Participating Institution Monitoring

Approximately 10% of the patients will be monitored on site, every 3 years. 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. Data entered at the website will be reviewed by the PI and study coordinator for any inconsistency. Queries as appropriate will be submitted to sites to clarify data. Submission of biologic specimens and imaging studies will be tracked on the study database.

12.4 Human Subjects Protection

12.4.1 Rationale for Subject Selection

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 MPNSTs develop in individuals with NF1, and we expect that approximately 50% of individuals enrolled will have NF1 associated MPNST, and 50% will have a sporadic MPNST.

12.4.2 Participation of Children

The treatment approach to MPNST is similar for children and adults. However, ganetespib alone nor in combination has been studied in children. Given the limited treatment options for refractory MPNST we plan to enroll patients ≥ 16 years old.

12.4.3 Evaluation of the Benefits and Risks/Discomforts

The primary risk to patients participating in this research study is from toxicity of the combination of ganetespib and sirolimus. The primary objective of this phase I/II trial is to determine the safety and tolerability and recommended doses of this combination and to then assess the clinical response rate in patients with refractory MPNST. 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 considered first line treatment by many oncologists for unresectable high-grade MPNSTs. The potential benefits from this therapy are disease stabilization, tumor shrinkage, and a reduction in symptoms caused by the cancer. Therefore, this protocol involves greater than minimal risk to the patients entered, but presents the potential for direct benefit to individual subjects.

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 NCI the FDA to satisfy regulatory requirements, and Synta Pharmaceuticals.

12.4.4 Risks/Benefits Analysis

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.

12.4.5 Consent and Assent Process and Documentation

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. The PI or an associate investigator on the trial will obtain consent from the patient or legal representative. The PI or associate investigator will meet with the patient to discuss the protocol treatment and alternative options in detail. It will be stated clearly that participation in the research study is voluntary and that participants can withdraw from the study without losing benefits they would otherwise be entitled to. The patient and family members will be encouraged to ask questions, and additional meetings to discuss the treatment options will be arranged as necessary.

12.4.6 Handling of Research Samples

This study is coordinated by SARC. Laboratory correlative studies are not mandatory and will be conducted as outlined in section 9. A detailed operations manual will be provide to each participating site, which will outline sample labeling, collection and processing. Once analyzed for studies outlined in this protocol any remaining samples will be stored at the site performing the analyses or at the SARC designated specimen bank until the study is complete, and the manuscript describing the study has been accepted for publication. The study will remain open and status reported to the IRB until all samples have been analyzed, reported, banked or destroyed. Unintentional loss or destruction of any samples will be reported to the IRB as part of annual continuing reviews. Any use of these samples for purposes not described in Section 9 will require prospective IRB review and approval. Tumor specimens will be sent to a SARC identified expert and will be delineated in the Operations Manual. Should tumor sample be left after completion of research studies described in the protocol, prospective approval from the appropriate IRB will be obtained prior to performing additional studies.

12.4.7 Handling of Patient Data

All patient data will be captured and maintained in a study specific database with password protected access. Data is entered using an assigned study subject identification number.

The data provided to those reviewing the results, for example the study statistician will include the subject identification numbers, but will not include patient identifiable data.

The research samples obtained on this study will only be sent using the study subject identification number, which can only be linked to the patient at a given institution by the treating physician.

All documentation that contains personal health information that may include patient identifiable information will be maintained at the site to preserve patient confidentiality.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

Phase I

In the phase I component, a conventional dose-escalation design is used. The initial starting dose of ganetespib is approximately 1 dose level below the recommended phase 2 weekly dose in combination with the recommended adult dose of sirolimus of 4 mg once daily. This will be followed by one dose escalation to the ganetespib weekly recommended phase 2 dose and sirolimus recommended adult dose. The total number of dose levels will likely only be 2, with the possibility of 2 dose de-escalations. Cohorts of 3 to 6 patients will be treated per dose level. At the MTD, the

cohort may be expanded to up to an additional 6 patients for further pharmacokinetic and tolerability experience. The MTD/Recommended dose will be defined as the dose level immediately below the level at which ≥33% of patients in a cohort experience a DLT based on toxicities observed in the first treatment cycle. In the phase 1 portion, this study is largely exploratory, precluding formal statistical comparisons of treatment groups. Toxicity and tolerability will be measured using CTCAE-4 and summarized by dose level. Grade ≥3 toxicities attributable to agents will be presented in frequency tables for each dose level.

Phase II

In the phase II component, the primary endpoint will be clinical benefit rate, which will be defined as a CR, PR, or stable disease ≥4 months. A completed trial of erlotinib in patients with refractory MPNST demonstrated a median time to progression of 48 days and progressive disease in the first response evaluation after completion of 2 treatment cycles in 19/20 patients [21]. Thus stable disease ≥ 4 months can be considered potentially beneficial and worthy of further investigation. An evaluable patient will be classified a responder (success) for the primary endpoint if the patient achieves a PR, CR or stable disease at ≥ 4 months as defined by WHO criteria. The target clinical benefit rate will be 25%, and a clinical benefit rate ≤ 5% will be considered uninteresting. Using Simon's optimal two-stage phase II design, the first stage will require 10 patients, with no further accrual if 0 of 10 respond. If ≥1/10 patients respond, accrual will continue until a total of 20 patients have been enrolled. If ≥3/20 patients respond, this combination will be considered of sufficient activity. Assuming the number of successes is binomially distributed, this design has a one sided alpha of 0.07 and a power of 88% for detecting a true success probability of at least 25% versus the null hypothesis success rate of 5% or less.

13.2 Definitions

Evaluable for Adverse Effects

Any patient who experiences DLT at any time during protocol therapy is considered evaluable for adverse effects. Patient without DLT who receive at least 85% of the prescribed sirolimus dose per protocols guidelines and had the appropriate toxicity monitoring performed are also considered evaluable for adverse effects. Patients who are not evaluable for adverse effects at a given dose level will be replaced.

Evaluable for Response

Any eligible patient who is enrolled and receives at least one dose of drug 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 or stable disease is confirmed, or 3) patient demonstrates a complete or partial response or stable disease per protocol criteria. The evaluation period for determination of best response will be 6 treatment cycles. All other patients will be considered non-responders

13.3 Sample Size/Accrual Rate

Phase I: 3 to 6 patients per cohort with 1 dose escalations (potential for 2 de-escalation). Thus a minimum of 6 patients to a maximum of 18 patients will be enrolled.

Phase II: 10 patients in first stage with an additional 10 patients in the second stage for a total of 20 patients. The maximum number of patients for entire study will be 38. It is expected that 15-25 patients be enrolled per year, and enrollment is expected to be completed in approximately 2.5 years.

13.4 Analysis of Secondary Endpoints

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

Pharmacokinetic analysis will be conducted using non-compartmental methods and estimated pharmacokinetic parameters including AUC, clearance, half-life and volume of distribution presented for each dose level using summary statistics (mean, standard deviation, median and range).

Analysis of secondary endpoints will be predominantly descriptive using primarily non-parametric analyses and will be interpreted as being exploratory and hypothesis generating. Results will be summarized with graphical analysis and correlations will be made if feasible.

Pharmacodynamic parameters including phospho-S6, phospho-S6, phosphorylated eIF2 alpha, Akt Phosphorylation, Hsp70, and G6PD will be evaluated in surrogate tissue (peripheral blood mononuclear cells) and tumor tissue (when feasible) at baseline and during treatment. Changes in these parameters will be correlated to radiographic response using logistic regression analysis.

Patient reported pain severity and impact of pain on activities of daily living will be summarized using descriptive statistics. Analysis of change in pain severity and pain interference over time may involve individual t-tests between 2 time points (or non-parametric Wilcoxon-Mann-Whitney tests) and repeated measures Analysis of Variance when appropriate. Correlations and chi-square analyses to compare pain severity and impact with objective tumor response and adverse events will be made if feasible.

Volumetric MRI: An automated method of volumetric analysis of plexiform neurofibromas in NF1 was recently developed, and is in use in clinical trials. This method is reproducible and allows detection of smaller changes in tumor size than conventional response criteria. Similar to plexiform neurofibromas, MPNSTs have a

complex shape (non spherical), and RECIST (1-dimensional)[22] or WHO (2-dimensional) criteria may have limited applicability. Volumetric MRI tumor analysis will be applied to MPNSTs as a tool for response assessment. Response evaluation using volumetric measurements will be compared to standard 2-dimensional response measurements (WHO criteria), and to 1-dimensional measurements (RECIST criteria). Spearman rank correlation will be used to describe the association.

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APPENDIX I: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work)
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed > 50% of the time. Capable of only limited self-care, confined in bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

APPENDIX II: Drug Interaction Table

Substrates of CYP3A4 or CYP2C19

Ganetespib is an inhibitor of CYP2C19 and CYP3A4. Concomitant medications that are substrates of CYP3A4 or CYP2C19 on this list are to be avoided. Refer to most recent ganetespib IB.

Examples of Medications that are Substrates of CYP3A4 or CYP2C19

Drug Class	Drug
CYP3A4 substrate examples	
Antibiotics (Macrolide)	clarithromycin
	erythromycin
Antifungals	ketoconazole
	itraconazole
Antiretrovirals	amprenavir
	indinavir
	lopinavir
	nevirapine
	ritonavir
	saquinavir
	nelfinavir
Benzodiazepines	midazolam
	alprazolam
	triazolam
Calcium channel blockers	diltiazem
	felodipine
	nifedipine
	verapamil
GI Agents	aprepitant

CYP2C19 substrate examples	
Anticonvulsants	phenytoin
Antifungals	voriconazole
Antiretrovirals	nelfinavir

APPENDIX III: PATIENT DIARY

OTHER MEDICATIONS TAKEN

If you take a daily medication (prescribed or otherwise), please use one line per drug and indicate the start and stop dates under the "Date(s) Taken" section (i.e., 6/2/09-6/5/09).

Drug Name	Dose	Dates Taken	Reason Taken

Study Participant Initials _____ Date _____

FOR OFFICE USE Staff Initials:	
Date Dispensed:	Date Returned:
# pills/tabs dispensed:	# pills/tabs returned:
# pills/tabs that should have been taken:	
Discrepancy Notes:	

SARC023 Participant Study Drug Diary

"Site Name"

Participant Identifier: _____

Cycle # _____ ***Cycle Start Date:*** _____

Your MD_____ Phone_____

Your RN_____ Phone_____

Study treatment instructions

Study treatment will be given in 28 day cycles.

Sirolimus

- Your dose of sirolimus is _____
- You will take sirolimus once per day every day of each 28 day cycle (except for very first dose in first cycle).
- Take each dose at the same time each day, preferably in the morning consistently with or without food.
- If you forget to take your dose at the scheduled time, you may still take it up to 6 hours after the normal scheduled time. If more than 6 hours have elapsed, you should skip the dose for that day. Mark the missed dose in this diary. Take the next day's dose as scheduled.
- If you vomit your dose of sirolimus, do not take that dose again. Mark the vomited dose in this diary. Take the next day's dose as scheduled.
- It is important that your dietary habits remain as consistent as possible throughout the study around the time you take sirolimus. You should avoid grapefruit, Seville oranges, or star fruit and the juices of these fruits and St. John's Wort while you are taking sirolimus, as these items may change how your body handles sirolimus.
- It is important that you maintain good oral hygiene (mouth care) while you are taking sirolimus to help prevent inflammation of the mouth tissues.
- Bring any unused sirolimus, all containers (empty and full), and this diary to each clinic visit. The study staff will make sure you have an adequate supply of sirolimus to take home at the end of each clinic visit.

SYMPTOMS/SIDE EFFECTS

Please record any side effects experienced during this cycle. Include the date the particular symptom started and when it ended. Please evaluate the severity of the symptom according to the following scale:

Mild: Awareness of sign or symptom; easily tolerated and did not affect ability to perform normal daily activities. Symptom did not require medication or therapeutic intervention.

Moderate: Significant discomfort which interfered with ability to perform normal daily activities. Symptom was easily resolved with at home medication or simple therapeutic intervention.

Severe: Marked discomfort with an inability to carry out normal daily activities. Symptom required new medication and/or therapeutic intervention in order to resolve.

Please Note: The severity should reflect the most severe level experienced during the time period.

Symptom	Start Date	End Date	Severity

Sirolimus Diary

	Sirolimus: For each dose, take _____. Please indicate the date, time, amount taken and any comments.		
	Date & Time	**Amount Taken**	**Comments**
Ex:	6/1/15 6:30 am	2 tablets	Vomited dose
Day 1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			

Sirolimus Diary Continued…

	Date & Time	Amount Taken	Comments
Ex:	6/1/15 6:30 am	2 tablets	Vomited dose
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			

APPENDIX IV: Documentation of findings of NF1

Demographic information:

DOB:		Sex:	
Mother's race:		Father's race:	
NF1 Inherited:	Yes: No:	NF1 sporadic:	Yes: No:

1	Exam Date	_ _ _ _ / _ _ / _ _ year month day
2	Height/length	_ _ _ . _ cm **or** _ ft _ _ . _ in Unknown
3	Head circumference	_ _ . _ cm _ _ . _ in Unknown
4	Number of café au lait (In pre-pubertal individuals, include CAL between 0.5 and 1.5cm)	None 1 2 3 4 5 6 or more Present, number unknown Unknown
5	Intertriginous Freckling	Absent Present Unknown
6	Subcutaneous neurofibromas	None 1 2 3 - 9 10-50 >50 Unknown

7	Cutaneous neurofibromas (Includes pendulous)	None 1 2 3 - 9 10-50 >50 Unknown
8	Plexiform neurofibroma - Location (Check as many as apply)	None Orbit Face Head/neck Trunk - dorsal Trunk - ventral Arm Leg Unknown
9	Paraspinal neurofibromas	Absent by scan Absent clinically *Present Unknown
10	Xanthogranulomas	Absent Present Unknown
11	Lisch nodules	Absent Present on slit lamp exam Possible Unknown
12	Proptosis	Absent Unilateral Bilateral Present, laterality unknown Unknown
13	Optic glioma	Absent by scan Absent clinically Present - asymptomatic Present - symptomatic Unknown
14	Seizures - type	None Febrile only Hypsarrhythmia Generalized Partial Multiple types Present - type unknown *Other

15	Hydrocephalus	Absent clinically Absent by scan Aqueductal stenosis Other non-communicating Communicating Present - type unknown Unknown
16	Intellectual Development	Normal Mildly Delayed Significant delay Unknown
17	Learning Problems	None Specific learning problems present Unknown
18	Hypertension	Absent Present Unknown
19	Congenital heart disease	Absent clinically Absent by special testing Aortic stenosis ASD Patent ductus arteriosus Pulmonic stenosis Tetralogy of Fallot VSD Other type of CHD Multiple types of CHD Possible CHD Unknown
20	Vascular anomalies	absent clinically *renal artery stenosis *arterial stenosis (non-renal) *moya moya *other unknown
21	Age puberty began	<10 years 10-15 years >15 years Not applicable Unknown
22	Dysmorphic features	No Yes Possible Unknown
23	Congenitally bowed tibia or pseudarthrosis	Absent clinically Absent radiographically Present Unknown

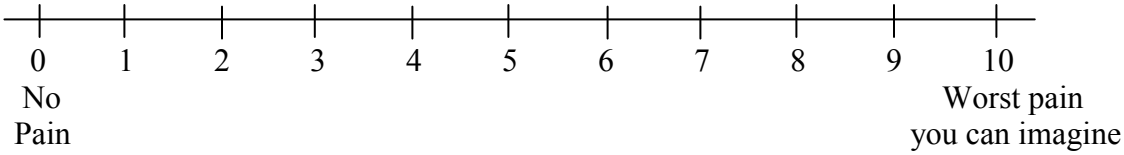
24	Dysplastic vertebrae	Absent clinically Absent radiographically Present Unknown
25	Scoliosis	Absent clinically Absent radiographically Present Unknown
26	Dysplastic sphenoid wing	Absent clinically Absent radiographically Present, bilateral Present, unilateral Present, laterality unknown Unknown
27	Neoplasm - type (Please check as many as apply)	None Carcinoma Ependymoma Glioma Leukemia Lymphoma Malignant peripheral nerve sheath tumour Meningioangiomatosis Meningioma Pheochromocytoma Sarcoma Schwannoma Malignancy present, type unknown Other

APPENDIX V: Pain Questionnaire

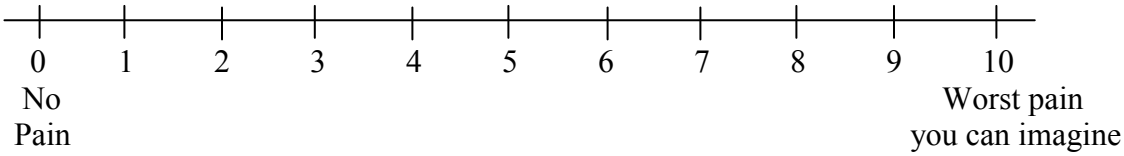
Protocol: SARC023
Patient Study ID: _____
Check One:
☐ Baseline ☐ Prior to cycle 3 ☐ Prior to cycle 5 ☐ Prior to cycle 9 ☐ Prior to cycle 13

Numeric Rating Scale (NRS-11) – Pain Intensity
Below are lines with numbers from 0 to 10 where 0 means no pain and 10 means the worst pain you can imagine.

1. For this question, please circle the **one number** that best describes your most important tumor pain at its **worst** during the past week. Please rate your pain for the same tumor throughout the study (specify the location of the tumor: _____).



2. For this question, please circle the **one number** that best describes your overall tumor pain at its **worst** during the past week.



Brief Pain Inventory (BPI) – Pain Interference
For the following questions, a "0" means that pain did not interfere with (get in the way of) the activity and a "10" means that pain complete interfered.

3. Circle the **one number** that describes how much, during the past week, tumor pain has interfered with your _____.

	Does not interfere										Completely interferes
1. General activity……....	0	1	2	3	4	5	6	7	8	9	10
2. Mood…….	0	1	2	3	4	5	6	7	8	9	10
3. Walking ability…….....	0	1	2	3	4	5	6	7	8	9	10
4. School, work, or chores……..……..	0	1	2	3	4	5	6	7	8	9	10
5. Relations with other people……..	0	1	2	3	4	5	6	7	8	9	10
6. Sleep…….....	0	1	2	3	4	5	6	7	8	9	10
7. Enjoyment of life……..	0	1	2	3	4	5	6	7	8	9	10

APPENDIX VI: Drugs with Risk of Torsades De Pointes

For the most current list of medications, please refer to the following website powered by azert.org: http://crediblemeds.org

Generic Name	Brand Names	Drug Class	Therapeutic Use
Amiodarone	Cordarone®, Pacerone®, Nexterone®	Anti-arrhythmic	Abnormal heart rhythm
Anagrelide	Agrylin®, Xagrid®	Phosphodiesterase 3 inhibitor	Thrombocythemia
Arsenic trioxide	Trisenox®	Anti-cancer	Leukemia
Azithromycin	Zithromax®, Zmax®	Antibiotic	Bacterial infection
Chloroquine	Aralen®	Anti-malarial	Malaria infection
Chlorpromazine	Thorazine®, Largactil®, Megaphen®	Anti-psychotic / Anti-emetic	Schizophrenia/ nausea
Citalopram	Celexa®, Cipramil®	Anti-depressant, SSRI	Depression
Clarithromycin	Biaxin®, Prevpac®	Antibiotic	Bacterial infection
Cocaine	Cocaine	Local anesthetic	Topical anesthetic
Disopyramide	Norpace®	Anti-arrhythmic	Abnormal heart rhythm
Dofetilide	Tikosyn®	Anti-arrhythmic	Abnormal heart rhythm
Dronedarone	Multaq®	Anti-arrhythmic	Atrial Fibrillation
Droperidol	Inapsine®, Droleptan®, Dridol®, Xomolix®	Anti-psychotic / Anti-emetic	Anesthesia adjunct, nausea
Erythromycin	E.E.S.®, Robimycin®, EMycin®, Erymax®, Ery-Tab®, Eryc Ranbaxy®, Erypar®, Eryped®, Erythrocin Stearate Filmtab®, Erythrocot®, E-Base®, Erythroped®, Ilosone®, MY-E®, Pediamycin®, Zineryt®, Abboticin®, Abboticin-ES®, Erycin®, PCE Dispertab®, Stiemycine®, Acnasol®, Tiloryth®	Antibiotic	Bacterial infection; increase GI motility
Escitalopram	Cipralex®, Lexapro®, Nexito®, Anxiset-E® (India), Exodus® (Brazil), Esto® (Israel), Seroplex®, Elicea®, Lexamil®, Lexam®, Entact® (Greece), Losita® (Bangladesh), Reposil® (Chile), Animaxen® (Colombia), Esitalo® (Australia), Lexamil® (South Africa)	Anti-depressant, SSRI	Major depression/ Anxiety disorders
Flecainide	Tambocor®, Almarytm®, Apocard®, Ecrinal®, Flécaine®	Anti-arrhythmic	Abnormal heart rhythm

Halofantrine	Halfan®	Anti-malarial	Malaria infection
Haloperidol	Haldol® (US & UK), Aloperidin®, Bioperidolo®, Brotopon®, Dozic®, Duraperidol® (Germany), Einalon S®, Eukystol®, Halosten®, Keselan®, Linton®, Peluces®, Serenace®, Serenase®, Sigaperidol®	Anti-psychotic	Schizophrenia, agitation
Ibutilide	Corvert®	Anti-arrhythmic	Abnormal heart rhythm
Methadone	Dolophine®, Symoron®, Amidone®, Methadose®, Physeptone®, Heptadon®	Opiate	Pain control, narcotic dependence
Moxifloxacin	Avelox®, Avalox®, Avelon®	Antibiotic	Bacterial infection
Ondansetron	Zofran®, Anset®, Ondemet®, Zuplenz®, Emetron®, Ondavell®, Emeset®, Ondisolv®, Setronax®	Anti-emetic	Nausea, vomiting
Pentamidine*	NebuPent®, Pentam®	Antibiotic	Pneumocystis pneumonia
Pimozide	Orap®	Anti-psychotic	Tourette's tics
Procainamide (Oral off US mkt)	Pronestyl®, Procan®	Anti-arrhythmic	Abnormal heart rhythm
Quinidine	Quinaglute®, Duraquin®, Quinact®, Quinidex®, Cin-Quin®, Quinora®	Anti-arrhythmic	Abnormal heart rhythm
Sevoflurane	Ulane®, Sojourn®	Anesthetic, general	Anesthesia
Sotalol	Betapace®, Sotalex®, Sotacor®	Anti-arrhythmic	Abnormal heart rhythm
Thioridazine	Mellaril®, Novoridazine®, Thioril®	Anti-psychotic	Schizophrenia
Vandetanib	Caprelsa®	Anti-cancer	Thyroid cancer

*** Injectable form (Inhaled formulation is allowed)**