SARC Protocol #: SARC016

TITLE: SARC016 Phase 2 study of the mTOR inhibitor everolimus in combination with bevacizumab in patients with sporadic and neurofibromatosis type 1 (NF1) related refractory malignant peripheral nerve sheath tumors

Sponsor: SARC (Sarcoma Alliance for Research through Collaboration)

Funding: Department of Defense

Supporters: Novartis and Genentech

Principal Investigator Clinical:
Brigitte C. Widemann, MD
National Cancer Institute
Pediatric Oncology Branch
10 Center Drive, room 1-5750
Bethesda, MD 20892-1101
Phone: 301-496-7387
Fax: 301-480-8871
E-mail: widemanb@mail.nih.gov

Medical Monitor/DoD Independent Research Monitor:
SARC Medical Officer
Scott Okuno, MD
Mayo Clinic
200 1st St SW
Rochester, MN 55905
Phone: 507-284-4849
Email: okuno.scott@mayo.edu

Database:
Medidata RAVE

Statistician:
Seth Steinberg, PhD
Head, Biostatistics and Data Management Section, Office of the Clinical Director
Center for Cancer Research, NCI
6116 Executive Blvd., Room 702
Bethesda, Maryland 20892
Phone: 301-496-9502
E-mail: steinbes@mail.nih.gov

Participating sites: SARC institutions, Department of Defense sponsored Neurofibromatosis Consortium institutions (full listing in Operations Manual)

IND #: IND Exempt (FDA acknowledgement and agreement on IND status received by SARC, March 18, 2011)

Protocol Type/Version # / Version Date: Phase 2 trial, version 3, 9 July, 2015

Roles and responsibility of study personnel:

Brigitte Widemann, MD: Principal investigator for the study

Scott Okuno, MD: SARC Medical Officer, providing SARC oversight to the conduct of this trial and ongoing medical monitoring; Independent Research Monitor for study

Denise Reinke, MS, NP, MBA: President and CEO, SARC

Vesna Milacic, PhD: Director of Research Project Management, SARC

Ndidi Onwudiwe, MS: Research Project Manager

Eva Dombi, MD: Volumetric MRI analysis of MPNSTs on protocol

Nalini Jayaprakash, MS, and Chand Khanna, DVM, PhD: Analysis of PD correlative studies

John Perentesis, MD: Investigator overseeing PD analysis of correlative studies

Seth Steinberg, PhD: Study Statistician

Karen Cichowski, PhD: Preclinical collaborator

Ludwine Messiaen, PhD: Genotyping

David Viskochil, MD: Serve as interface between NF1 centers and sarcoma centers; facilitate referral of patients not affiliated with NF1 centers

Lee Helman, MD, Brigitte Widemann, MD, Christina Annunziata, MD, Melinda Merchant, MD, PhD, John Glod, MD, PhD,: Enrollment of patients and patient care responsibilities at the NIH

Donna Bernstein, RN, Andy Gillespie, RN: Research nurses for study at the NIH

Joanne Derdak, C.R.N.P.**:** Clinical care of patients enrolled on this study at the NCI

GLOSSARY OF ABBREVIATIONS

4E-BP1	4E-binding protein
ADR	Adverse Drug Reaction
AE	adverse event
ALP	Alkaline phosphatase
ALT (SGPT)	Alanine aminotransferase/glutamic pyruvic transaminase/Serum glutamic-pyruvic transaminase
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate aminotransferase/glutamic oxaloacetic transaminase/Serum glutamic-oxaloacetic transaminase
ATC	Anatomical Therapeutic Chemical classification system
AUC	Area under the plasma-concentration time curve
CI	Confidence interval
Cmax	Maximum plasma concentration
CGH	Comparative Genomic Hybridization
CR	Complete Response or clinical research
CRF	Case report/Record form
CRO	Contract Research Organization
CT	Computer tomography
CTC	Common toxicity criteria
CV	Coefficient of Variation
CYP3A4	CytochromeP450 3A4 isoenzyme
DLT	Dose Limiting Toxicity
EC_{50}	plasma concentration associated with half-maximal effect
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
eIF-4E	Eucariotic Initiation Factor 4E
eIF2 alpha	Eucariotic translation initiation factor 2 alpha
ESFT	Ewing Sarcoma Family of Tumors
FDG-PET	Fluorine-18-2-fluoro-Deoxy-D-Glucose Positron Emission Tomography

FISH	Fluorescence in situ hybridization
FKBP-12	FK506-binding protein 12
G-CSF	Granulocyte colony-stimulating factor
GF	Growth factor
HBV	Hepatitis B virus
HBcAb	Hepatitis B core antibodies
HBsAb	Hepatitis B surface antibodies
HBsAg	Hepatitis B surface antigen
HBC	Hepatitis C Virus
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HDL	High-density lypoproteins
HER	Human Epidermal Receptor
HSCT	Hematopoietic Stem Cell Transplant
huMAb IGF-1R	Human monoclonal antibody against the human insulin-like growth factor 1 receptor; RO4858696
HUVECS	human umbilical endothelial cells
IEC	Independent Ethics Committee
IGF1-R	Insulin-like Growth Factor 1 Receptor
IHC	Immunohistochemistry
INN	International Non-proprietary Name
INR	International Normal Ratio
IR	Insulin receptor
IRB	Institutional Review Board
k_{eo}	Equilibration rate constant
LC-MS	liquid chromatography method with mass spectrometry
LDL	Low-density lypoproteins
LFT	Liver function tests
LLN	lower limit of normal
LLQ/LLOQ	Lower limit of quantification
LVEF	Left Ventricular Ejection Fraction
MAPK	Mitogen Activated Protein Kinase
mRNA	messenger Ribonucleic acid

MPNST	Malignant peripheral nerve sheath tumor
mTOR	mammalian Target of Rapamycin
NCI-CTC	National Cancer Institute-Common toxicity Criteria
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Events
NF1	Neurofibromatosis type 1
NIH/NCI	National Institutes of Health/National Cancer Institute
nM	nano-molar
NOAEL	No observed adverse event level
NSCLC	Non-small cell lung cancer
ORR	Objective response rate
OS	overall survival, osteosarcoma
P-AKT	phosphor-AKT, a human oncogenic protein
PCR	Polymerase Chain Reaction
PD	Progressive disease or Pharmacodynamics
PET	Proton emission tomography
PFS	progression free survival
P-gp	P-glycoprotein
PI3K	Phosphoinositide 3-kinase
PK	Pharmacokinetics
PK/PD model	Pharmacokinetic/pharmacodynamic model
PR	Partial response
PS	Performance Status
PT/PTT	prothrombin time
PTEN	Phosphatase and Tensin homolog deleted on chromosome 10
p-PTEN	Phosphorylated form of PTEN, a human tumor suppressor
RBC	red blood cell count
REB	Research Ethics Board
RECIST	Response Evaluation Criteria in Solid Tumors
RR	response rate
S6K1	S6 kinase 1
SAE	serious adverse event

SCLC	Small cell lung cancer
SD	Stable disease
SF	Shortening Fraction
STAT3	Signal Transducer and Activator of Transcription 3
STS	Soft tissue sarcoma
TGI	Tumor growth inhibition
TK	Tyrosine kinase
TNM	primary tumor/regional lymph nodes/distant metastasis
TSC2	Tuberous Sclerosis Complex 2
TTP	Time to Tumor Progression
TUNNEL	Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin Nick End Labeling
ULN	upper limit of normal
UPC ratio	Urine Protein Creatinine ratio
VEGF	Vascular Endothelial Growth Factor
WBC	total white blood cell count
WHO	World Health Organization

TREATMENT OUTLINE:

Patients with NF1 related or sporadic refractory MPNST will receive 28 day cycles of everolimus + bevacizumab until disease progression or unacceptable toxicity for a maximum of 2 years.

Everolimus will be administered at a dose of 10 mg/dose once daily on a continuous dosing schedule.

Bevacizumab will be administered at a dose of 10 mg/kg IV every 2 weeks (day 1 and 15).

Response evaluation (WHO criteria) will be performed before every other treatment cycle (3, 5, 7, 9, etc).

SYNOPSIS:

Primary objective:

- To determine the clinical benefit rate (complete response, partial response, and stable disease at ≥ 4 months using WHO criteria) of everolimus in combination with bevacizumab for patients with chemotherapy refractory sporadic or neurofibromatosis type 1 (NF1) associated malignant peripheral nerve sheath tumor (MPNST)
- To evaluate the toxicity and safety of everolimus in combination with bevacizumab in individuals with MPNST

Secondary objectives:

- To evaluate the spectrum of germline NF1 mutations in individuals with NF1 associated MPNSTs
- To explore the relationship between response to everolimus in combination with bevacizumab and the presence of NF1 mutations or NF1 inactivation in MPNST tumor samples
- To explore differences in the response rate to everolimus in combination with bevacizumab in individuals with sporadic and NF1 associated MPNST
- To assess preliminary correlations of radiographic response and progression with changes in pharmacodynamic parameters including S6K1 (p70 S6 kinase 1), eIF4E, eIF2 alpha VEGF, VEGFR, Akt phosphorylation, and markers of cell metabolism in peripheral blood specimens
- To evaluate the utility of three-dimensional MRI (3D-MRI) analysis in comparison to 1-dimensional and 2-dimensional measurements to more sensitively monitor response to everolimus in combination with bevacizumab

Hypothesis and rationale:

The NF1 tumor suppressor regulates mammalian target of rapamycin (mTOR) pathway activation, which appears to be critical for the progression of MPNSTs. The mTOR inhibitor sirolimus halts progression of MPNSTs for prolonged time periods in a murine genetically engineered NF1 MPNST model and in a NF1 and sporadic mouse MPNST xenograft model. Angiogenesis contributes to the progression of MPNST, and in the transgenic mouse model MPNSTs become ultimately resistant to treatment with rapamycin. The development of resistance is associated with re-vascularization and upregulation of vascular endothelial growth factor (VEGF). Preliminary preclinical data in the transgenic NF1 MPNST mouse model demonstrate prolonged survival for mice treated with rapamycin plus sunitinib, which in part, mediates anti-tumor activity by inhibition of angiogenesis, compared to mice treated with rapamycin or sunitinib alone (unpublished data K. Cichowski). These findings provide the rationale for our proposed phase 2 trial of the mTOR inhibitor everolimus in combination with the angiogenesis inhibitor bevacizumab. This trial will determine the activity of everolimus in combination with bevacizumab in refractory MPNST and allow validation of the genetically engineered MPNST mouse model by preliminarily evaluating pathways

associated with response and disease progression. In addition, this trial will serve as a model for future combination trials of targeted agents for MPNST.

Trial design:
This is a two-stage phase 2 clinical trial with the objective to assess activity of the mTOR inhibitor everolimus in combination with the angiogenesis inhibitor bevacizumab in patients with sporadic and NF1 associated MPNSTs refractory after ≥ 1 prior cytotoxic chemotherapy regimen. The diagnosis of NF1 will be made based on clinical criteria. The primary endpoint is clinical benefit rate (PR, CR or stable disease at ≥ 4 months). All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be evaluable for response. Everolimus will be administered at a dose of 10 mg po once daily on a continuous dosing schedule (28 days = 1 cycle). Bevacizumab will be administered IV at a dose of 10 mg/kg/dose every 14 days.

Initially enrollment will be limited to patients ≥ 18 years old. Patients will be regularly monitored for everolimus and bevacizumab toxicity and response. Success will be defined as PR, CR, and SD at ≥ 4 months using WHO response criteria. A two-stage design will be used, and a clinical benefit rate of ≥ 25% will be defined as success ruling out a < 5% clinical benefit rate.

Numbers of subjects:
Fifteen patients will be enrolled on the first stage, with no further accrual if no successes (PR, CR, and SD at ≥ 4 months) are observed within the first 15 patients. If at least one success is observed, accrual will continue until a total of 25 evaluable patients have been enrolled. If at least four successes are observed in the 25 patients everolimus in combination with bevacizumab will be considered active in that it would be consistent with a 25% success rate.

Target population:
Individuals ≥ 18 years of age with adequate organ function and unresectable or metastatic MPNST who experienced progression after ≥ one prior cytotoxic chemotherapy regimen will be eligible. Patients have to be able to swallow tablets. The protocol will be amended to allow enrollment of pediatric patients as soon as an ongoing pediatric phase I trial of everolimus and bevacizumab has determined the pediatric phase II dose.

Length of study:
Patients will be able to remain on treatment for a maximum of 2 years as long as they do not experience progressive disease or unacceptable toxicity. It is expected that 10-15 patients per year will be enrolled, and enrollment is expected to complete within approximately 1 to 2 years depending on the number of responses observed in the initial stage.

Study drugs:
- Everolimus as 2.5 mg, 5 mg, and 10 mg tablets
- Bevacizumab: Supplied in vials containing 400 mg bevacizumab

Dosing and administration:

- Everolimus will be administered once daily at a dose of 10 mg on a continuous dosing schedule (1 cycle = 28 days)
- Bevacizumab will be administered every 14 days at a dose of 10 mg/kg IV (1 treatment cycle =28 days)

Efficacy evaluations:
Response evaluations (WHO) with appropriate imaging studies (MRI/CT) will be performed before the beginning of every other treatment cycle (3, 5, 7, 9, etc.).

Safety:
History and physical examination including vital signs will be regularly performed during treatment with everolimus and bevacizumab. Laboratory studies will include CBC with differential, fasting glucose and lipid panel, and a comprehensive chemistry panel.

Molecular analysis:

- Frozen tumor samples, if available, will be evaluated for NF1 mutation/inactivation.
- Blood samples will be obtained for NF1 genotyping in patients with a clinical diagnosis of NF1.

Pharmacodynamics:
Peripheral blood samples will be collected prior to and serially during treatment with everolimus for S6K1 (p70 S6 kinase 1), eIF4E, eIF2 alpha, VEGF, VEGFR, Akt phosphorylation, and markers of cell metabolism.

Statistical Analysis:
An evaluable patient will be classified 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 < 5% will be considered uninteresting. Using a two-stage phase 2 design with 5.0% as the value for a one-sided alpha, the first stage will require 15 patients, with no further accrual if 0 of 15 respond. If $\geq$ 1/15 patients respond accrual will continue until a total of 25 patients have been enrolled. If $\geq$ 4/25 patients respond, everolimus with addition of bevacizumab will be considered active in that it would be consistent with a 25% clinical benefit rate. Assuming the number of successes is binomially distributed, this design has a one sided alpha of 5.0% and a power of 90% 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 objectives:

- To determine the clinical benefit rate (complete response, partial response, and stable disease at ≥ 4 months using WHO criteria) of everolimus in combination with bevacizumab for patients with chemotherapy-refractory sporadic or neurofibromatosis type 1 (NF1) associated malignant peripheral nerve sheath tumor (MPNST)
- To evaluate the toxicity and safety of this everolimus in combination with bevacizumab in individuals with MPNST

1.2 Secondary objectives:

- To evaluate the spectrum of germline NF1 mutations in individuals with NF1 associated MPNSTs
- To explore the relationship between response to everolimus in combination with bevacizumab and the presence of NF1 mutations or NF1 inactivation in MPNST tumor samples
- To explore differences in the response rate to everolimus in combination with bevacizumab in individuals with sporadic and NF1 associated MPNST
- To assess preliminary correlations of radiographic response and progression with changes in pharmacodynamic parameters including S6K1 (p70 S6 kinase 1), eIF4E, eIF2 alpha VEGF, VEGFR, Akt phosphorylation, and markers of cell metabolism in peripheral blood specimens
- To evaluate the utility of three-dimensional MRI (3D-MRI) analysis in comparison to 1-dimensional and 2-dimensional measurements to more sensitively monitor response to everolimus in combination with bevacizumab

2. BACKGROUND:

2.1 Malignant peripheral nerve sheath tumors

Malignant peripheral nerve sheath tumors (MPNSTs), (also called neurogenic sarcomas, malignant schwannomas, neurofibrosarcomas) are soft tissue sarcomas, which show nerve sheath differentiation and are associated with a high risk of local recurrence and hematogenous metastasis[1]. They account for 10% of all soft tissue sarcomas, and half of these malignancies arise in patients with neurofibromatosis type 1 (NF1)[2].

Neurofibromatosis 1 (NF1) is a common autosomal dominant, progressive genetic disorder with an incidence of 1:3000 (> 80,000 persons affected in The United States). Neurofibromin, the NF1 gene product, contains a domain with significant homology to *ras* GTPase-activating proteins (GAP)[3]. The *ras* proteins are integral in cell signaling pathways, and activation of *ras* leads to cell proliferation. GAPs catalyze the hydrolysis of *ras*-GTP (the active form of *ras*) to *ras*-GDP and lead to *ras* inactivation. Patients with NF1 have decreased levels of neurofibromin,

which is associated with an activated *ras*-GTP status. Lack of functional neurofibromin can lead to dysregulated *ras* and tumorigenesis. NF1 is characterized by diverse, progressive cutaneous, neurological, skeletal and neoplastic manifestations. Patients with NF1 have an increased risk of developing tumors of the central and peripheral nervous system including plexiform neurofibromas (27%) optic gliomas (15-20%), pheochromocytomas (1%), and MPNSTs (5%)[4,5].

MPNSTs occur in about 2-5% of patients with NF1 compared with an incidence of 0.001% in the general population[1,6]. However, in a large population based longitudinal study the lifetime risk of developing a MPNST in NF1 was 8-13%[4]. In patients with NF1, the majority of MPNSTs arise in a previously clinically detectable plexiform neurofibroma, but they may develop in absence of preexisting tumors[6-8]. Early diagnosis of MPNSTs is crucial, as only complete surgical resection has been shown to be curative. However, the diagnosis of MPNSTs in patients with NF1 is difficult to establish, because clinical indicators of malignancy (mass and pain) may also be features of benign plexiform neurofibromas. Contrast enhanced MRI[9], and new imaging modalities like ^{18}FDG-PET[10] are being evaluated for their potential to differentiate benign plexiform neurofibromas from MPNSTs. The most frequent sites of metastasis of MPNSTs are lung, liver, brain, soft tissue, bone, regional lymph nodes, and retroperitoneum[6].

Staging of MPNST: As other soft tissue sarcomas (STS), MPNSTs are most commonly staged using the American Joint Committee on Cancer staging system[11]. In this system, four tumor grades are designated: Well differentiated (G1), moderately differentiated (G2), poorly differentiated (G3), and undifferentiated (G4). Poor prognostic factors for MPNST include: Tumor size > 5 cm, deep location, high grade (G3, G4), presence of distant metastases at time of diagnosis, and radiation induced MPNST. In addition, based on a postoperative nomogram for the 12-year sarcoma specific death, the presence of MPNSTs carries the highest risk for sarcoma specific death compared to other histologic types of STS[12].

Epidemiology and outcome: The epidemiology and outcome of NF1 associated MPNSTs may be different from sporadic MPNSTs, in that several studies report a younger age at diagnosis and worse prognosis for NF1 associated MPNSTs[4,13]. Reasons for the potentially worse outcome of NF1 associated MPNSTs are unknown. Two studies indicate that NF1 associated MPNSTs may develop more frequently as central, non-extremity lesions, which could impact on outcome, as central lesions are less amenable to surgery[6,14]. A recently published large retrospective review of 126 individuals with MPNST treated on Italian and German studies between 1975 and 1998 described that response to chemotherapy was lower in NF1-associated MPNSTs (3 of 17 individuals responded, = 17.6%), compared to sporadic MPNSTs (26 of 47 responded = 55.3%). Details regarding

the chemotherapy agents used were not provided[13]. Similarly, in a more recent study Ferrari et al reported described a 5-year overall survival of 11% for 27 children with NF1 associated MPNST compared to 48% for 44 patients with sporadic MPNST[2]. However, it is not clear that NF1-related MPNSTs have a worse prognosis when adjusted for the known prognostic features. A recently published study, for example, describes excellent survival in MPNSTs with no difference between sporadic and NF1 associated tumors[15]. Potential reasons for the favorable survival compared to other reports are not discussed in this manuscript, but 70% of the MPNSTs described were extremity lesions compared to 30% in central location. Recently completed 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].

Molecular biology of MPNSTs: Surgical specimens and cell lines from patients with MPNSTs and NF1 demonstrate complete inactivation of NF1 and high levels of *ras* activity[17-19]. Benign plexiform neurofibromas also demonstrate complete inactivation of *NF1* with evidence suggesting that the Schwann cells represent the neoplastic element[20-22], therefore additional genetic alterations and abnormalities of other biochemical pathways likely contribute to progression to malignancy. Immunohistochemical analysis and molecular studies have implicated p53, EGFR, p16, and p27 as potential contributors to malignant transformation in peripheral nerve sheath tumors. Gene expression profiling of human MPNSTs showed expression of EGFR in 16 of 42 (38%) of human[16]. Further highlighting the unique pathogenesis of this tumor type, both *NF1* deletions and homozygous *p16* deletions appear to be relatively restricted to MPNSTs in comparison to other spindle cell sarcomas with overlapping morphologic features[22]. In addition, microarray analysis of MPNSTs recently identified topoisomerase IIα (TOP2A) as the most overexpressed gene in MPNSTs compared to benign neurofibromas, and TOP2A expressing tumors were associated with poor cancer specific survival and presence of metastasis[23].

Treatment of adult soft tissue sarcomas (STS) and MPNSTs: Treatment of MPNST follows the treatment of other adult soft tissue sarcomas. Only complete surgical resection has been shown to be curative, and it remains the cornerstone of therapy[11,24]. The goal of surgery is to resect the MPNST with wide negative margins. However, the local recurrence rate of MPNST is high, and ranges from 32-65%[25]. Radiotherapy is used in situations where the sarcoma is not amenable to surgical resection, but, when used as primary treatment, large doses are needed and the control rate is only 30-60%. Clinical trials have demonstrated that external beam radiation or brachytherapy in addition to limb sparing surgery improve local control in patients with soft tissue sarcomas[26,27]. Adjuvant radiotherapy is thus recommended to improve local control for intermediate to high grade lesions > 5 cm after a marginal excision[1,26,28].

The role of chemotherapy for MPNSTs has not been defined to date. Only doxorubicin, dacarbazine, and ifosfamide were consistently associated with response rates of 20% or more in adults with soft tissue sarcomas and the combination of ifosfamide and doxorubicin has produced response rates as high as 46%[29]. However, adjuvant doxorubicin-based chemotherapy did not demonstrate statistically significant improvement in overall survival [29]. In patients with advanced, metastatic soft tissue sarcomas doxorubicin and ifosfamide have the highest activity. The overall response rate for single agent doxorubicin was 19% (range 16-27%), and for ifosfamide response rates of 36% and 28% have been reported[29,30].

Responses to chemotherapy have been described in children and adults with MPNSTs[31-33], but the response rate of MPNSTs to chemotherapy is unknown. They are thought to have intermediate chemosensitivity, less responsive than synovial sarcoma, but more responsive than refractory diseases such as alveolar soft part sarcoma[34].

A recent retrospective review of individuals with MPNSTs described less response to chemotherapy for individuals with NF1 associated MPNSTs compared to sporadic MPNSTs[13].

Currently a Department of Defense sponsored, SARC coordinated, phase 2 clinical trial is ongoing with the primary objective to prospectively determine the response rate of high grade unresectable chemotherapy naïve MPNST to standard chemotherapy agents (doxorubicin, ifosfamide, and etoposide) used to treat pediatric and adult patients. As the response to chemotherapy may differ between sporadic and NF1 associated tumors, this trial stratifies for the presence of sporadic versus NF1 associated MPNST. Depending on its outcome this trial may serve in the future as a model for the addition of targeted therapies for MPNST. In addition Children's Oncology Group study ARST0332 (Risk-based treatment for non-rhabdomyosarcoma soft tissue sarcomas (NRSTS) in patients under 30 years of age, PI Sheri Spunt) is ongoing, and enrolls patients with newly diagnosed NF1 and sporadic MPNST. Treatment on this protocol includes, depending on the treatment arm, surgery, radiation, and chemotherapy with doxorubicin and ifosfamide. This study will thus also provide additional information regarding treatment and outcome of sporadic and NF1 associated MPNST.

With increasing knowledge of the molecular biology of MPNST there is a strong rationale for the development of targeted treatment approaches for MPNST[35]. A recently completed trial of the EGFR inhibitor erlotinib for individuals with refractory MPNST based on EGFR expression in MPNSTs demonstrated lack of activity with 19/20 evaluable patients developing progressive disease at the time of the first response evaluation after completion of 2 treatment cycles[36]. This trial demonstrated the feasibility of conducting histology specific phase 2 trials for MPNSTs. Similarly sorafenib, a raf kinase and RTK inhibitor, recently completed

evaluation in several sarcoma strata including refractory and metastatic MPNSTsma[37]. Sixteen patients with MPNST were enrolled. No partial or complete responses were observed, and progression free survival (PFS) was 1.7 months (range, 1.3 to infinity), overall survival was 4.9 months (range 3 months to infinity), and the Kaplan-Meier estimates for percent PFS at 3 and 6 months were 25% and 8%, respectively. In another recently reported phase II trial of dasatinib (SARC009), which included a refractory MPNST stratum, none of 12 evaluable patients had a PR or CR, or stable disease at 4 months, and the median time to progression was 35 days[38]. Finally, in a phase II trial of imatinib for refractory sarcomas, including an MPNST stratum, of 6 patients with MPNST, who received imatinib, 4 had progressive disease at 2 months, 1 was removed from the trial for toxicity early, and one withdrew from the study early[39]. These studies highlight the need for the development of more effective targeted treatments for MPNST and provide justification for inclusion of stable disease at 4 months as a criterion for success.

Recently, NF1was identified as a regulator of the mTOR pathway, and based on the data described in Section 2.3, we are proposing a phase 2 trial of the mTOR inhibitor everolimus in combination with the angiogenesis inhibitor bevacizumab for refractory MPNST.

2.2 Study agents: everolimus (Afinitor®/Votubia®) and bevacizumab (Avastin®)

Everolimus is a novel oral derivative of rapamycin. Everolimus has been in clinical development since 1996 as an immunosuppressant in solid organ transplantation and has obtained marketing authorization (Certican®) for prophylaxis of rejection in renal and cardiac transplantation in a number of countries, including the majority of the European Union. Everolimus has been in development for patients with various malignancies since 2002. Everolimus was first approved under the trade Afinitor® for patients with advanced renal cell carcinoma (RCC) and is now approved in the EU and several other countries, as well as by the US Food and Drug Administration (FDA). The FDA granted accelerated approval in November 2010 for patients with subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis (TSC) who require therapeutic intervention but are not candidates for curative surgical resection. Approval in patients with SEGA was based on a Phase II open label study in which 75% of patients experienced reductions of $\geq$ 30%, and 32.1% experienced reductions of $\geq$ 50%. Range of time on study was reported as 4.7 to 47.1 months. It is noteworthy in the TSC setting in patients with angiomyolipoma (AML) that an international multicentre Phase III placebo controlled study demonstrated a 41.8% AML response rate among everolimus treated patients versus a 0% response rate among the placebo treated patients ($p < 0.0001$). Median time to angiomyolipmoma progression was 11.4 months in the placebo arm and was not reached in the everolimus arm, leading to regulatory approval in patients with AML. Everolimus was later approved in 2011 for patients with advanced

neuroendocrine tumors of the pancreas (pNET) based on the results of a randomized, double-blind, multi-centre phase III study that showed a statistically significant clinical benefit over placebo with a median PFS of 11.0 months vs 4.6 months, resulting in a 65% risk reduction in PFS.

Everolimus is being investigated as an anticancer agent based on its potential to act:

- Directly on the tumor cells by inhibiting tumor cell growth and proliferation
- Indirectly by inhibiting angiogenesis leading to reduced tumor vascularity (via potent inhibition of tumor cell HIF-1 activity, VEGF production and VEGF-induced proliferation of endothelial cells). The role of angiogenesis in the maintenance of solid tumor growth is well established, and the mTOR pathway has been implicated in the regulation of tumor production of proangiogenic factors as well as modulation of VEGFR signaling in endothelial cells.

At weekly and daily schedules and at various doses explored, everolimus is generally well tolerated. The most frequent adverse events (incidence ≥ 10% and suspected to be related to treatment by the investigator) were infections, stomatitis, pyrexia, acneiform dermatitis, diarrhea, acne, cough, hypertriglyceridemia, and decreased white blood cell count. The only grade 3 adverse reactions were infections (single cases of sinusitis, pneumonia, tooth infection, and viral bronchitis), and single cases of stomatitis and decreased white blood cell count. No Grade 4 adverse events were reported.

mTOR pathway and mechanism of action

At a cellular and molecular level everolimus acts as a signal transduction inhibitor. Everolimus selectively inhibits mTOR (mammalian target of rapamycin), a key and a highly conservative serine-threonine kinase, which is present in all cells and is a central regulator of protein synthesis and ultimately cell growth, cell proliferation, angiogenesis and cell survival. mTOR is the only currently known target of everolimus[40].

mTOR is downstream of PI3K/AKT pathway, a pathway known to be dysregulated in a wide spectrum of human cancers (e.g. through loss/mutation of the PTEN negative regulator; through PI3K mutation/amplification; through AKT/PKB overexpression/overactivation; through modulation of TSC1/TSC2 tumor suppressors). In addition, activation of the PI3K/AKT/mTOR pathway is frequently a characteristic of worsening prognosis through increased aggressiveness, resistance to treatment and progression.

The main known functions of mTOR include the following[40,41]:

- mTOR functions as a sensor of mitogens, growth factors and energy and nutrient levels, facilitating cell-cycle progression from G1 to S phase in appropriate growth conditions.
- The PI3K-mTOR pathway itself is frequently activated in many human cancers, and oncogenic transformation may sensitize tumor cells to mTOR inhibitors.
- Through inactivating eukaryotic initiation factor 4E binding proteins (4E-BP1) and activating the 40S ribosomal S6 kinases (i.e., p70S6K1), mTOR regulates translation of important massages, including those encoding the HIF-1 proteins, c-myc, ornithine decarboxylase, and cyclin D1, as well as ribosomal proteins themselves.
- The activation of mTOR pathway leads to the increased production of pro-angiogenic factors (i.e., VEGF) in tumors and to tumor, endothelial and smooth muscle cell growth and proliferation.
- The regulation of mTOR signaling is complex and involves positive regulators, such as AKT that phosphorylate and inactivate negative regulators such as the Tuberous Sclerosis Complex (TSC1/TSC2).

mTOR is represented by two structurally and functionally distinct multiprotein signaling complexes, mTORC1 (mTOR complex 1, rapamycin sensitive) and mTORC2 (mTOR complex 2, rapamycin insensitive).

mTORC1 is mainly activated via the PI3 kinase pathway through AKT (also known as PKB, protein kinase B) and the tuberous sclerosis complex (TSC1/TSC2)[41]. Activated AKT phosphorylates TSC2, which lead to the dissociation of TSC1/TSC2 complex, thus inhibiting the ability of TSC2 to act as a GTPase activating protein. This allows Rheb, a small G-protein, to remain in a GTP bound state and to activate mTORC1. AKT can also activate mTORC1 by PRAS40 phosphorylation, thereby relieving the PRAS40-mediated inhibition of mTORC1[42].

mTORC2 (mTOR complex 2) is activated through a currently unknown mechanism, possibly by receptor tyrosine kinase (RTK) signaling[42]. It has been suggested that mTORC2 phosphorylates and activates a different pool of AKT that is not upstream of mTORC1. PHLPP phosphatase plays a role of a negative regulator. mTORC2 is rapamycin insensitive and is required for the organization of the actin cytoskeleton[43].

mTORC1-mediated signaling is subject to modulation by the macrocyclic lactone rapamycin and its derivatives, such as everolimus. Once these agents bind to the 12 kDa cytosolic FK506-binding protein immunophilin FKBP12, the resulting rapamycin-FKBP12 complexes bind to a specific site near the catalytic domain of mTORC1 and inhibit phosphorylation of mTOR substrates. As a consequence, downstream signaling events involved in regulation of the G1 to S-phase

transition are inhibited. This mechanism is thought to be responsible for the immunosuppressive effects of rapamycin as well as its putative antineoplastic activity[44]. As many cancers are characterized by dysregulation of G1 transit (for example, overexpression of cyclin or cyclin-dependent kinases), inhibition of mTOR becomes an intriguing target for inducing cytostasis[41].

Preclinical studies

Preclinical investigations have demonstrated that everolimus is a potent inhibitor of the proliferation of a broad range of human tumor cell lines *in-vitro* with IC50s ranging from sub/low nM to μM concentrations, concentrations capable of being reached in patients at the doses used in clinical trials.

Everolimus was shown to have activity in human tumor cell lines originating from lung, breast, prostate, colon, kidney, melanoma and glioblastoma. Everolimus was also shown to have activity in human pancreatic neuroendocrine cells, where induction of apoptosis was reported[45], as well as in acute myeloid leukemia cell[46], adult T-cell leukemia cells[47], diffuse large B cell lymphoma cells[48], pancreatic tumor cells[49], ovarian cancer cells[50] and hepatocellular carcinoma cells[51].

As a single agent, everolimus inhibited proliferation in three mantle cell lymphoma cell lines (Jeko1, SP49 and NCEB1) approximately 40–65% compared to control cells. This was associated with G1 cell-cycle arrest and reduced phosphorylation of the mTOR downstream target, 4E-BP1[52].

In a clonogenic assay using cells derived from 81 patient-derived tumor xenografts never cultured *in vitro* (11 human tumor types with 3 to 24 tumors each: bladder, colon, gastric, NSCLC [adeno, squamous epithelium and large cell], SCLC, breast, ovary, pancreatic, renal, melanoma, and pleuramesothelioma), everolimus inhibited colony formation in a concentration-dependent manner. In addition, normal hematopoetic stem cells were insensitive to everolimus, with an IC50 about 15 fold higher than the tumor lines.

Everolimus also inhibits the proliferation of human umbilical vein endothelial cells (HUVECS), with particular potency against VEGF-induced proliferation. The inhibition of endothelial proliferation and antiangiogenic activity of everolimus was confirmed *in vivo*, as everolimus selectively inhibited VEGF-dependent angiogenic response. Mice with primary and metastatic tumors treated with everolimus showed a significant reduction in blood vessel density when compared to controls at well tolerated doses. Additionally, activity in a VEGF-impregnated s.c. implant model of angiogenesis and reduced vascularity (vessel density) of everolimus-treated tumors (murine melanoma) provided evidence of *in vivo* effects of angiogenesis.

Everolimus also inhibits tumor growth *in-vivo* in xenografted, syngeneic and orthotopic animal models, residing longer in tumor tissue than in plasma and

demonstrating high tumor penetration in a rat pancreatic tumor model. These effects occurred within the dose range of 2.5 to 10 mg/kg p.o. daily. Typically, the antitumor activity of everolimus monotherapy was that of reduction of tumor growth rates rather than producing regressions or stable disease.

Everolimus, administered p.o., was a potent inhibitor of tumor growth and well tolerated in:

- s.c. mouse xenograft model, established from a variety of tumor cell lines of diverse histotypes (NSCLC, pancreatic, colon, melanoma, epidermoid), including a Pgp170 overexpressing multi-drug resistant tumor line
- in a series of low-passage tumor xenografts established directly from human tumor material, maintained only *in vivo* and considered highly predictive of therapeutic outcome in patients. These included breast (5 lines), colorectal (9 lines), gastric (3 lines), lung (22 lines including adenocarcinomas, epidermoid cell, large cell and small cell histotypes), melanoma (6 lines), ovarian (4 lines), pancreatic (3 lines) and renal (6 lines)
- in two syngeneic models (CA20948 rat pancreatic, B16/Bl6 mouse orthotopic melanoma)

Taken together, these data indicate the broad antiproliferative potential of everolimus.

It is not clear which molecular determinants predict responsiveness of tumor cells to everolimus. Molecular analysis has revealed that relative sensitivity to everolimus *in vitro* correlates with the degree of phosphorylation (activation) of the AKT/PKB protein kinase and the S6 ribosomal protein. PTEN status alone may not be predictive of everolimus relative *in vitro* sensitivity, however in some cases (i.e., GBM) there is also a correlation with PTEN status.

In preclinical models, the administration of everolimus is associated with reduction of protein phosphorylation in target proteins downstream of mTOR, notably phosphorylated S6 (pS6) and p4E-BP1, and occasionally with an increase in phosphorylation AKT (pAKT).

Preclinical safety

In safety pharmacology studies, everolimus was devoid of relevant effects on vital functions including the cardiovascular, respiratory and nervous systems. Everolimus had no influence on QT interval prolongation. Furthermore, everolimus showed no antigenic potential. Although everolimus passes the blood-brain barrier, there was no indication of relevant changes in the behavior of rodents, even after single oral doses up to 2000 mg/kg or after repeated administration at up to 40 mg/kg/day. Based on these findings, the potential of everolimus to affect vital functions in patients is considered to be low.

Everolimus is considered to have no genotoxicity or carcinogenicity potential.

All significant adverse events observed in preclinical toxicology studies with everolimus in mice, rats, monkeys and minipigs were consistent with its anticipated pharmacologic action as an antiproliferative and immunosuppressant and at least in part reversible after a 2 or 4-week recovery period with the exception of the changes in male reproductive organs, most notably testes. Ocular effects (lenticular disorders) observed in rats were not observed in any other species and are considered to be a species-specific disorder.

More pre-clinical information is provided in the Investigator Brochure[53].

Clinical experience

Everolimus Pharmacokinetics: everolimus is rapidly absorbed with a median T_{max} of 1-2 hours. The bioavailability of the drug is believed to be 11% or greater. The $AUC_{0-\tau}$ is dose-proportional over the dose range between 5 to 70 mg in the weekly regimen and 5 and 10 mg in the daily regimen. C_{max} is dose-proportional between 5 and 10 mg for both the weekly and daily regimens. At doses of 20 mg/week and higher, the increase in C_{max} is less than dose-proportional. The coefficient of variation between patients is approximately 50%.

Trough levels (24 hour post-dose) correlate well with $AUC_{0-\tau}$ at steady-state during daily administration.

In whole blood, at a daily dose of 10 mg, about 20% of everolimus is confined in plasma with 26% being unbound. The remaining 80% is sequestered in blood cells.

Everolimus is extensively metabolized in the liver and eliminated in the bile. Major metabolites are inactive. Elimination half-life is approximately 30 hours. The clearance of everolimus is approximately halved in patients with mild-moderate hepatic impairment (Child-Pugh Class A or B), while renal impairment has little or no impact on the pharmacokinetics of everolimus.

Age, weight and gender in the adult population do not affect the pharmacokinetics of everolimus to a clinically relevant extent. The clearance of everolimus is reduced in children.

Pharmacokinetic characteristics are not notably different between Caucasian and Japanese subjects, whereas in Black patients population pharmacokinetic studies have shown an average 20% higher clearance.

A high-fat meal altered the absorption of everolimus with 1.3 hour delay in T_{max}, a 60% reduction in C_{max} and a 16% reduction in AUC.

Everolimus is a substrate of CYP3A4 and a substrate and a moderate inhibitor of the multi-drug efflux pump P-glycoprotein (P-gP, MDR1, ABCB1). Hence, its

metabolism is sensitive to drugs which modify these enzymes (substrates, inducers, or inhibitors of these enzymes). Competitive inhibition could occur when everolimus is combined with drugs which are also CYP3A4 or P-glycoprotein substrates.

Appendix II lists examples of clinically relevant CYP3A inhibitors and inducers.

Please refer to section 3.2.14 for more information on the concomitant use of CYP3A4 inhibitors/inducers and other medications.

More information on everolimus pharmacokinetics is provided in the Investigator Brochure[53].

Everolimus Pharmacodynamic studies

Pharmacokinetic/pharmacodynamic modeling based on inhibition of the biomarker p70S6 kinase 1 [S6K1][54] in peripheral blood mononuclear cells suggests that 5-10 mg daily should be an adequate dose to produce a high-degree of sustained target inhibition[55]. Furthermore, molecular pharmacodynamic (MPD) studies, using immunocytochemistry (IHC) in biopsied tumor tissue, assessed the degree of inhibition and its duration for pS6, p4E-BP1 and pAKT expression with the daily and weekly dosing. There was high inhibition of the downstream markers S6K1 and 4E-BP1 at 5 mg/day, which was complete at 10 mg/day, while preliminary results suggest increase in pAKT expression with maximal effect at 10 mg daily[56].

More information is provided in the Investigator Brochure[53].

Clinical studies with everolimus

Everolimus has been investigated as a component of multi-drug immunosuppression in solid organ transplantation since 1996 and was approved for the indication of prophylaxis of organ rejection in adult patients receiving an allogeneic renal or cardiac transplant on 8 Jul 2003 by the European Union under the trade name of Certican®. The most frequent adverse drug reactions in this context are highly specific to the transplant context. However, certain events are generalizable, most notably myelosuppression, skin disorders and increases in blood lipid levels.

Everolimus (everolimus) was approved by the FDA in March 2009 for the treatment of advanced renal cell carcinoma for patients with advanced renal cell carcinoma (RCC) after failure of treatment with sunitinib or sorafenib. It was also approved as previously noted in 2010 for patients with SEGA with TC who require therapeutic intervention but are not candidates for curative surgical resection and in 2011 for patients with pNET. It is also FDA approved for lung neuroendocrine tumors and Waldenström's macroglobulinemia/ lymphoplasmacytic lymphoma.

Everolimus has been in development for patients with cancer since 2002. Everolimus 5 mg and 10 mg tablets were recently approved under the trade name Afinitor® for patients with advanced renal cell carcinoma in the US, EU and several other countries and is undergoing registration in other regions worldwide.

Phase I dose escalating studies, exploratory Phase I/II studies with everolimus as single agent or in combination with other anti-cancer agents, Phase II/III studies of everolimus in indications, and Phase III double-blind studies are contributing to the extensive database. Approximately 18,730 patients have been treated with everolimus as of 30-September 2011:

- 9,528 patients in Novartis-sponsored clinical trials
- 2,559 patients in the single patients use IND program
- 6,638 patients in investigator-sponsored studies
- In addition, healthy volunteer subjects and hepatically impaired non-oncology subjects have participated in the clinical pharmacology studies

As of 23 Nov 2011, there are a total of 8 Phase III trials ongoing in the indications mRCC (1), advanced GEP-NET (3), breast cancer (1), DLBCL (1) and, Hepatocellular carcinoma (1).

Recent approvals of everolimus were based upon a Phase III, international, multicenter randomized, double-blind, placebo-controlled study [C2240] in patients with metastatic renal cell carcinoma (mRCC) whose disease had progressed despite prior treatment with VEGFR-TKI (vascular endothelial growth factor receptor tyrosine kinase inhibitor) therapy. Progression-free survival (PFS) assessed *via* a blinded, independent central review, was the primary endpoint. Secondary endpoints included safety, objective tumor response

In the pivotal, Phase III study, which included patients with advanced renal cell carcinoma, the most common adverse reactions (incidence ≥ 10%) were stomatitis, rash, fatigue, asthenia, diarrhea, anorexia, nausea, mucosal inflammation, vomiting, pneumonitis, cough, peripheral edema, infections, dry skin, epistaxis, pruritus, and dyspnea. The most common grade 3-4 adverse reactions (incidence ≥ 2%) were infections, stomatitis, fatigue, and pneumonitis. Non-infectious pneumonitis is a class effect of rapamycin derivatives, including everolimus and some of these cases have been severe and on rare occasions, fatal outcomes have been observed. Everolimus has immunosuppressive properties and may predispose patients to bacterial, fungal, viral or protozoan infections, including infections with opportunistic pathogens. Localized and systemic infections, including pneumonia, other bacterial infections, invasive fungal infections, such as aspergillosis or candidiasis and viral infections including reactivation of hepatitis B virus, have been described in patients taking

everolimus. Some of these infections have been severe (e.g. leading to respiratory or hepatic failure) and occasionally have had a fatal outcome.

The most common laboratory abnormalities (incidence ≥ 50%) were anemia, hypercholesterolemia, hypertriglyceridemia, hyperglycemia, lymphopenia, and increased creatinine. The most common grade 3/4 laboratory abnormalities (incidence ≥ 3%) were lymphopenia, hyperglycemia, anemia, hypophosphatemia, and hypercholesterolemia. Deaths due to acute respiratory failure (0.7%), infection (0.7%), and acute renal failure (0.4%) were observed on the everolimus arm. The rates of treatment-emergent adverse reactions resulting in permanent discontinuation were 7% and 0% for the everolimus and placebo treatment groups, respectively.

Overall, safety data available from completed, controlled and uncontrolled studies are consistent with the aforementioned findings of the Phase III trial. Everolimus is generally well tolerated at weekly and daily dose schedules. The safety profile is characterized by manageable adverse events (AEs). These AEs are generally reversible and non-cumulative.

For more information on known undesirable effects of everolimus refer to Section 8.1.6.

A phase 1 study of everolimus in pediatric patients with refractory cancers was recently performed, and determined a dose of 5 mg/m^2/dose once daily on a continuous dosing schedule as the recommended phase 2 dose[57]. Of 26 patients enrolled, 18 were assessable for toxicity, and the toxicity profile was similar to that in adults. Dose-limiting toxicities at the 6.5 mg/m^2 dose level were mucositis (n=1), diarrhea (n=1), and LALT elevation (n=1). The pharmacokinetics of everolimus in children were similar to those in adults. For more information on known undesirable effects of everolimus refer to Section 8.1.6.

Impaired renal function is not expected to influence everolimus pharmacokinetics based on the fact that ≤ t percent of radioactivity was excreted in urine in the mass balance study in maintenance renal transplant patients CP Study. According to the IB, no dose adjustment is necessary for patients with renal impairment. Further detailed information regarding everolimus clinical development, safety and efficacy is provided in the Investigator Brochure[53].

Bevacizumab (rhuMAb) is a recombinant humanized anti-VEGF monoclonal antibody composed of human IgG1 framework regions and antigen-binding complementarily-determining regions from a murine monoclonal antibody (muMAb VEGF A.4.6.1) which blocks the binding of human VEGF to its receptors. Approximately 93% of the amino acid sequence, including most of the antibody framework, is derived from human IgG_1, and ~7% of the sequence is derived from the murine antibody.

Bevacizumab has been approved by the FDA as first line therapy in combination with chemotherapy for treatment of patients with metastatic colorectal cancer or unresectable, locally advanced, recurrent or metastatic, non-squamous non-small cell lung cancer because of improved overall survival in the patients receiving combination therapy as compared to receiving chemotherapy alone. These lines of evidence demonstrate that bevacizumab is clinically effective when combined with chemotherapy.

Preclinical studies: In cynomolgus monkeys, twice weekly IV treatments with bevacizumab (doses of 2, 10 and 50 mg/kg) for 4, 13 or 26 weeks were well tolerated, with no overt signs of acute toxicity[58]. Animals with open growth plates showed physeal dysplasia as well as focal to diffuse chondroid necrosis and linear fissuring of the cartilaginous growth plate. Females treated with 10-50mg/kg twice weekly had decreased ovarian and uterine weights, which were associated with absence of corpora lutea. These findings were expected, considering the known role of VEGF in formation of the corpora lutea and of the growing bone[59]. A further study using a similar treatment regimen, in the recovery period the physeal dysplasia and ovarian and uterine changes induced by rhuMAb VEGF were partially reversible. No antibodies against bevacizumab were detected.

Clinical studies in adults: Two phase-1 studies have been performed. Study AVF0737g was a dose escalation trial of single and multiple intravenous (IV) administration of rhuMAb in patients with advanced malignancies. Five dose levels were evaluated (0.1, 0.3, 1.0, 3.0, and 10mg/kg). rhuMAb VEGF was administered as a 90-minute infusion on days 0, 28, 35 and 42[60]. Study AVF0761g evaluated multiple doses of rhuMAb VEGF 3 mg/kg weekly for up to 8 weeks in combination with one of three cytotoxic chemotherapy regimens (5-fluorouracil/leucovorin, carboplatin/paclitaxel, or doxorubicin) in subjects with advanced solid malignancies[61]. rhuMAb VEGF was administered as eight weekly doses of 3mg/kg.

In both studies, rhuMAb VEGF appeared to be well tolerated. In study AVF0737g, 3 of 25 patients treated experienced tumor-related hemorrhagic events, possibly related to the administration of rhuMAb VEGF. In two cases the event was considered serious: an intracranial hemorrhage (at an occult cerebral metastasis) in a patient with hepatocellular carcinoma and bleeding at the tumor site in a 38-year-old woman with a slowly progressing sarcoma of the thigh. No patient in AVF0761g reported serious bleeding. No dose limiting toxicity was reached in either study. No antibodies to rhuMAb VEGF were detected after therapy in either study. Three subjects from each study subsequently enrolled in the extension study.

Pharmacokinetics: In study AVF0737g, the pharmacokinetics of rhuMAb VEGF appeared to be linear for doses ≥ 1mg/kg with a half-life of approximately 15

days. Comparable pharmacokinetic data was seen in study AVF0761g. Co-administration of rhuMAb and cytotoxic chemotherapy did not appear to result in a change in the systemic concentration of the cytotoxic agents.

Phase 2 Clinical Studies:

Study	Population	Study Treatment	rhuMAb VEGF Dosing Regimen
AVF0757g	Stage IIIB or IV non-small cell lung cancer	Carboplatin/ paclitaxel ± rhuMAb VEGF	7.5mg or 15mg/kg every 3 weeks until disease progression
AVF0780g	Metastatic colorectal cancer	5-FU/leucovorin ± rhuMAb VEGF	5mg or 10mg/kg every other week until disease progression
AVF0776g	Relapsed metastatic breast cancer	Single-agent rhuMAb VEGF	3, 10 or 20 mg/kg every other week over a 168-day treatment period or until disease progression
AVF0775g	Hormone-refractory prostate cancer	Single-agent rhuMAb VEGF	10mg/kg every other week over a 168-day treatment period or until disease progression

In Study AVF0780g, patients with metastatic colon cancer were treated with either 5-FU/leucovorin (500mg/m^2 5-FU and 500mg/m^2 leucovorin administered weekly for six weeks, with courses repeated every eight weeks) alone or in combination with rhuMAb VEGF 5mg/kg or 10mg/kg every two weeks.

Response rates were 17%, 40% (p=.03) and 24% (p=.23), respectively. A prolonged time to disease progression was seen in patients treated with rhuMAb VEGF 5mg/kg in combination with chemotherapy (9.0 months p=.005) compared with those who received rhuMAb VEGF 10mg/kg (7.2 months p= .217), or chemotherapy alone (5.2 months)[62].

The anti-VEGF antibody Bevacizumab (rhuMAb) was evaluated as a single agent in several malignancies including CRPC[61]. In a Phase 2 trial 15 patients with CRPC were treated with 10 mg/kg every 14 days. Results of the study showed that though the drug was tolerated well, there were no significant objective responses.

Toxicities: The following are the important toxicities. The detailed information can be seen in Section 8.2.8.

- ***Infusion-Related Reactions:*** Infusion reactions with bevacizumab were uncommon (< 3%) and rarely severe (0.2%). Infusion reactions may include rash, urticaria, fever, rigors, hypertension, hypotension, wheezing, or hypoxia. Currently, there is no adequate information on the safety of retreatment with bevacizumab in patients who have experienced severe infusion-related reactions.

- ***Hemorrhage:*** Life threatening hemorrhage was seen in a Phase 1 trial (AVF0737g) in the form of an intracranial hemorrhage (at a cerebral metastasis) in a patient with hepatocellular carcinoma and in the Phase 2 study (AVF0757g) in the form of massive hemoptysis or hematemesis. There were 6 life-threatening hemorrhages among 66 patients receiving rhuMAb VEGF-treated patients of which four of these events were fatal. An analysis of possible risk factors for life-threatening bleeding identified squamous cell histology as a risk factor (4 of 6 bleeds occurred in patients with squamous cell histology whereas only 13 of 66 rhuMAb VEGF-treated patients had squamous histology). A number of investigations were performed on two of the patients with pulmonary hemorrhage and in eight patients in AVF0780g receiving rhuMAb VEGF including platelet count, prothrombin time, activated prothrombin time, fibrinogen, bleeding time, euglobulin clot lysis, d-dimer, alpha2-antiplasmin, PFA-100 (a platelet function assay) and these were all within normal range (Novotny,W. Genentech Inc. personal communication).

  Overall, grade 3 and 4 bleeding events were observed in 4.0% of 1,132 patients treated with bevacizumab in a pooled database from eight phase 1, II, and III clinical trials in multiple tumor types (bevacizumab Investigator Brochure, October 2005). The hemorrhagic events that have been observed in bevacizumab clinical studies were predominantly tumor-associated hemorrhage (see below) and minor mucocutaneous hemorrhage.

Mucocutaneus hemorrhage: Across all bevacizumab clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of patients treated with bevacizumab. These were most commonly NCI-CTC Grade 1 epistaxis that lasted less than 5 minutes, resolved without medical intervention and did not require any changes in bevacizumab treatment regimen. There have also been less common events of minor mucocutaneous hemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

Tumor-associated hemorrhage: Major or massive pulmonary hemorrhage/hemoptysis has been observed primarily in patients with NSCLC. In a phase 2 study in NSCLC, 6 cases of life-threatening (4 fatal) hemoptysis were reported among 66 patients treated with bevacizumab and chemotherapy[63]; squamous cell histology was identified as the risk factor. In the phase III trial in non-squamous NSCLC (E4599), the rate of Grade ≥ 3 pulmonary hemorrhage was <1% in the control arm (carboplatin/paclitaxel) versus 2.3% in the chemotherapy plus bevacizumab arm (10/427 patients, including 7 deaths).

Gastrointestinal hemorrhages, including rectal bleeding and melaena have been reported in patients with colorectal cancer, and have been assessed as tumor-associated hemorrhages. In the pivotal phase 3 trial in advanced colorectal cancer, the rate of GI hemorrhage (all grades) was 24% in the IFL/bevacizumab arm compared to 6% in the IFL arm; grade 3-4 hemorrhage was 3.1% for IFL/bevacizumab and 2.5% for IFL.

Serious tumor associated bleedings have also been observed in patients with pancreatic cancer, gastric cancer, CNS metastases, hepatoma, or varices treated with bevacizumab.

- ***Thrombosis:*** Both venous and arterial thromboembolic (TE) events, ranging in severity from catheter-associated phlebitis to fatal, have been reported in patients treated with bevacizumab in the colorectal cancer trials and, to a lesser extent, in patients treated with bevacizumab in NSCLC and breast cancer trials.

  The risk of arterial thromboembolic events (ATE) is increased with bevacizumab therapy; such events included cerebral infarction, transient ischemic attack (TIA), myocardial infarction (MI) and other peripheral or visceral arterial thrombosis. A pooled analysis of five randomized studies showed a two-fold increase in these events (3.8% vs. 1.7%). ATE led to a fatal outcome in 0.8% patients with bevacizumab (vs. 0.5% without bevacizumab). The rate of cerebrovascular accidents (including TIA) was 2.3% vs. 0.5%, and the rates of MI 1.7% vs. 0.7%. Certain baseline characteristics, such as age and prior arterial ischemic events, appear to confer additional risk[64]. In patients > 65 years treated with bevacizumab and chemotherapy, the rate of ATE was approximately 8.5%.

Aspirin is a standard therapy for primary and secondary prophylaxis of arterial thromboembolic events in patients at high risk of such events, and the use of aspirin ≤ 325 mg daily was allowed in the five randomized studies discussed above. Use of aspirin was assessed routinely as a baseline or concomitant medication in these trials, though safety analyses specifically regarding aspirin use were not preplanned. Due to the relatively small numbers of aspirin users and arterial thromboembolic events, retrospective analyses of the ability of aspirin to affect the risk of such events were inconclusive. However, similarly retrospective analyses suggested that the use of up to 325 mg of aspirin daily does not increase the risk of grade 1-2 or grade 3-4 bleeding events. Further analyses of the effects of concomitant use of bevacizumab and aspirin in colorectal and other tumor types are ongoing.

Venous thromboembolism (VTE), including deep venous thrombosis, pulmonary embolism and thrombophlebitis: In the Phase III pivotal trial in metastatic CRC, there was a slightly higher rate of venous thromboembolism (VTE) in patients treated with chemotherapy + bevacizumab compared with chemotherapy alone (19% vs. 16%). The incidence of NCI-CTC Grade ≥ 3 VTEs in one NSCLC trial (E4599) was higher in the bevacizumab-containing arm compared to the chemotherapy control arm (5.6% vs. 3.2%).

In clinical trials across all indications, the overall incidence of VTEs ranged from 2.8% to 17.3% in the bevacizumab-containing arms compared to 3.2% to 15.6% in the chemotherapy control arms. The use of bevacizumab with chemotherapy does not substantially increase the risk of VTE compared with chemotherapy alone. However, patients with mCRC who receive bevacizumab and experienced VTE may be at higher risk for recurrence of VTE.

- ***Hypertension:*** Hypertension is common in patients treated with bevacizumab. The incidence of hypertension (all grade) is 20-30% across trials, with a mean increase of +5.5mmHg to +8.4mmHg for systolic pressure, or +4.1mmHg to +5.4mmHg for diastolic pressure. Incidence of grade 3 (hypertension requiring initiation of, or increase in, hypertensive medications) ranges from 7.8 to 17.9%. Grade 4 hypertension (hypertensive crisis) occurred in up to 0.5% of bevacizumab-treated patients.

  Hypertension associated with bevacizumab can generally be controlled with routine oral drugs while bevacizumab is continued. However, incidents of hypertensive crisis with encephalopathy, including reversible posterior leukoencephalopathy syndrome (RPLS, see below), or cardiovascular sequelae have been rarely reported. Blood pressure (BP) should be closely monitored during bevacizumab therapy and the goal of

BP control should be consistent with standard medical practice[65]. Bevacizumab therapy should be suspended in the event of uncontrolled hypertension.

- ***Gastrointestinal Perforation:*** GI perforations and/or fistula were rare but occurred at an increased rate in bevacizumab-containing therapies. The majority of such events required surgical intervention and some were associated with a fatal outcome. In the pivotal phase III trial in CRC (AVF2107), the incidence of bowel perforation was 2% in patients receiving IFL/bevacizumab and 4% in patients receiving 5-FU/bevacizumab compared to 0.3% in patients receiving IFL alone. GI perforation has also been reported in non-CRC tumors (e.g. gastric/esophageal, pancreatic and ovarian cancers) or nonmalignant conditions such as diverticulitis and gastric ulcer. GI perforation should be included in the differential diagnosis of patients on bevacizumab therapy presenting with abdominal pain or rectal/abdominal abscess.
- ***Fistula:*** Fistula formations, including events resulting in death, have been observed in patients receiving bevacizumab in clinical studies and post-marketing reports. Fistulae in the GI tract are common (1-10% incidence) in patients with certain metastatic tumors such as colorectal cancer or cervical, but uncommon (0.1-1%) or rare (0.01-0.1%) in other indications. In addition, fistulae that involve areas other than the GI tract have also been observed (e.g. tracheoesophageal, bronchopleural, urogenital, biliary). Events were reported at various time points during treatment, ranging from 1 week to > 1 year following initiation of bevacizumab, with most events occurring within the first 6 months of therapy.
- ***Wound Healing Complications:*** Bevacizumab delays wound healing in rabbits, and it may also compromise or delay wound healing in patients. Bowel anastomotic dehiscence and skin wound dehiscence have been reported in clinical trials with bevacizumab.

  The appropriate interval between surgery and initiation of bevacizumab required to avoid the risk of impaired wound healing has not been determined. Across metastatic CRC trials, at least 28 days must have elapsed following major surgery before bevacizumab could be initiated; data suggested initiation of bevacizumab 29-60 days following surgery did not appear to increase the risk of wound healing complications compared to those treated with chemotherapy alone.

  The optimal interval between termination of bevacizumab and subsequent elective surgery has not been determined. In the pivotal study in CRC, among patients who underwent major surgery while on study therapy, there was an increased rate of significant post-operative bleeding or wound healing complications in the IFL + bevacizumab arms vs. IFL alone -10% (4/40) vs. 0% (0/25)-[66].

Decisions on the timing of elective surgery should take into consideration the half-life of bevacizumab (average 21 days, range 11-50 days).

If patients receiving treatment with bevacizumab require elective major surgery, it is recommended that bevacizumab be held for 4–8 weeks prior to the surgical procedure. Patients undergoing a major surgical procedure should not begin/restart bevacizumab until 4 weeks after that procedure (in the case of high-risk procedures such as liver resection, thoracotomy, or neurosurgery, it is recommended that chemotherapy be restarted no earlier than 6 weeks and bevacizumab no earlier than 8 weeks after surgery).

- ***Proteinuria:*** Proteinuria has been seen in all bevacizumab studies to date, ranging in severity from mild asymptomatic increase in urine protein (incidence of about 38%) to rare instances of either grade 3 proteinuria (> 3.5gm/24 hour urine) (3%) or nephrotic syndrome (1.4%). Pathologic findings on renal biopsies in two patients showed proliferative glomerulonephritis. The risk of proteinuria may be higher in patients with advanced RCC or history of hypertension. There is also evidence from dose-finding trials that the rate of proteinuria may be dose related. Proteinuria will be monitored by urine protein level using urine analysis dipstick, or the urine may be sent to the institution's lab for processing.

- ***Congestive Heart Failure (CHF):*** The risk of left ventricular dysfunction may be increased in patients with prior or concurrent anthracycline treatment. In phase III trials in metastatic breast cancer (AVF 2119g) in which all patients had received prior anthracyclines, CHF or cardiomyopathy were reported in 3% in the bevacizumab + capecitabine arm compared to 1% in the capecitabine-only arm[67]. In a phase III trial of patients with previously untreated metastatic breast cancer (E2100), the incidence of LVEF decrease (defined as NCICTC Grade 3 or 4) in the paclitaxel + bevacizumab arm was 0.3% versus 0% for the paclitaxel alone arm.

  In phase II study of 48 patients with refractory acute myelogenous leukemia treated with cytarabine, mitoxantrone, and bevacizumab, 5 cases of cardiac dysfunction (CHF or decreases to < 40% in left ventricular ejection fraction, including AML trial) were reported. All but one of these subjects had significant prior exposure to anthracyclines as well.

  Two additional studies investigated concurrent administration of anthracyclines and bevacizumab. In 21 patients with inflammatory breast cancer treated with neoadjuvant docetaxel, doxorubicin (cumulative doses at 240 mg/m2), and bevacizumab, no patients developed clinically apparent CHF; however, patients had asymptomatic decreases in LVEF to < 40% (78). In a small phase II study in patients with soft tissue sarcoma, 2/17 patients treated with bevacizumab and high-dose doxorubicin (75 mg/m^2) developed CHF (one Grade 3 event after a cumulative doxorubicin

dose of 591 mg/m^2, one Grade 4 event after a cumulative doxorubicin dose of 420 mg/m^2); an additional 4 patients had asymptomatic decreases in LVEF[68].

Patients receiving anthracyclines or with prior exposure to anthracyclines should have a baseline MUGA or ECHO with a normal ejection fraction.

- ***Reversible Posterior Leukoencephalopathy Syndrome (RPLS), Posterior Reversible Encephalopathy Syndrome (PRES), or similar leukoencephalopathy syndrome:*** RPLS/PRES are clinical syndromes related to vasogenic edema of the white matter and have rarely reported in association with bevacizumab therapy (< 1%). Clinical presentations may include altered mental status, seizure, visual disturbance or cortical blindness, with or without associated hypertension. MRI scans are required for diagnosis. Typical findings are vasogenic edema (enhanced intensity in T2 and FLAIR sequences on non-contrast MRI) predominantly in the white matter of the posterior parietal and occipital lobes, and less frequently, in the anterior distributions and the gray matter.

  RPLS/PRES is potentially reversible, but timely correction of the underlying causes, including control of BP and interruption of the offending drug, is important in order to prevent irreversible tissue damage. The safety of reinitiating bevacizumab therapy in patients previously experiencing RPLS is not known[69].

- ***Neutropenia:*** In the phase III trial with IFL +/- bevacizumab in colorectal cancer, grade 3-4 neutropenia was 21% with bevacizumab + IFL vs. 14% with IFL (grade 4 neutropenia was 3% vs. 2%). Increased rates of severe neutropenia, febrile neutropenia, or infection with severe neutropenia (including some fatalities) have been observed in patients treated with some myelotoxic chemotherapy regimens plus bevacizumab. In a phase III in NSCLC, carboplatin and paclitaxel + bevacizumab arm was associated with increased rate of grade 4 neutropenia (27% vs. 17%), febrile neutropenia (5.4% vs. 1.8%), and infection with neutropenia (4.4% vs. 2.0%) with three fatal cases[70].
- ***Fertility and Pregnancy:*** Clinical data are lacking regarding the immediate or long-term effect of bevacizumab on fertility and pregnancy. However, bevacizumab is known to be teratogenic and detrimental to fetal development in animal models. In addition, bevacizumab may alter corpus luteum development and endometrial proliferation, thereby having a negative effect on fertility. As an IgG1, it may also be secreted in human milk. Therefore, fertile men and women on bevacizumab studies must use adequate contraceptive measures and women should avoid breast feeding. The duration of such precautions after discontinuation of bevacizumab should take into consideration the half-life of the agent (average 21 days, ranging from 11 to 50 days).

- ***Immunogenicity:*** As a therapeutic protein, there is a potential for immunogenicity with bevacizumab. With the currently available assay with limited sensitivity, high titer human anti-bevacizumab antibodies have not been detected in approximately 500 patients treated with bevacizumab.

Reference should be made to the product labeling of bevacizumab. Specific caution and clinical alertness should be exercised for patients receiving the agent.

Clinical Studies in Children: A phase 1 trial of bevacizumab as single agent has been completed for children with refractory solid tumors. In this study, doses from 5 mg/kg to 15 mg/kg every 2 weeks were well tolerated[71]. Eighteen of 21 children completed ≥ 1 treatment cycle (median cycle #3, range 1-16 cycles). Interestingly, the typical adverse events seen in adults; hypertension, proteinuria, arterial thromboembolic events, hemorrhage, congestive heart failure (CHF), gastrointestinal perforations, wound healing complications, reversible posterior leukoencephalopathy syndrome and fistula formation, were not seen in children. Bony toxicity was not observed, but cumulative toxicity could not be evaluated given the fairly short treatment duration with bevacizumab. Based on these results, phase 2 studies at doses similar to adults were recommended.

Combination studies of everolimus and bevacizumab: Multiple clinical trials combining everolimus with bevacizumab are ongoing in adults (clinicaltrials.gov). In these studies everolimus is typically administered at a dose of 10 mg po/day on a continuous doing schedule, and bevacizumab is administered at a dose of 10 mg/kg IV every 14 days or 15 mg/kg IV every 21 days.

On a phase II trial of bevacizumab (10 mg IV every 2 weeks) and everolimus (10 mg orally once daily) in patients with advanced renal cell carcinoma 80 patients were enrolled (50 untreated, 30 previously treated). Bevacizumab/everolimus showed activity in both groups. The median PFS in previously untreated and previously treated patients were 9.1 and 7.1 months, respectively. ORR (30% and 23%). The combination was well-tolerated by most patients, with a toxicity profile as expected based on the known toxicities of both agents. Grade-3/4 proteinuria was more frequent than expected (25%) and led to treatment discontinuation in 6 patients (unpublished, information provided by Novartis).

In another phase II trial of bevacizumab 15 mg/kg Q 21 days + everolimus 10 mg daily for patients with metastatic melanoma, 57 patients enrolled received a median of 4 cycles (range: 1-14+). Seven patients (13%) had major responses, 30 patients (53%) had objective decrease in the size of measurable lesions. The median PFS and OS were 4 months and 8.6 months, respectively. The regimen was well tolerated, and 43% were alive after 12 months follow-up (unpublished data, information provided by Novartis).

One pediatric phase 1 trial combining bevacizumab 10 mg/kg IV every 14 days with everolimus 4 mg/m^2/dose (starting dose level 1) and 5 mg/m^2/dose (dose level 2) once daily po on a continuous dosing schedule is ongoing. The recommended phase 2 dose on this trial has not been determined yet (personal communication Lisa McGregor, study PI). Therefore pediatric patients will not be initially eligible for trial participation.

2.3 Rationale

Role of Mammalian target of rapamycin (mTOR) in cell proliferation:
Mammalian Target of rapamycin (mTOR) is a serine/threonine kinase which is regulated by the phosphoinositol 3 kinase (PI3K)/Akt signaling pathway. Evidence suggests mTOR acts as a master switch of numerous cellular processes, including cellular catabolism and anabolism, cell motility, angiogenesis, and cell growth[72]. mTOR integrates two of the most important signals involved in the regulation of cell growth: growth factors and nutrients. Growth factors, such as insulin and insulin-like growth factor, and nutrients, such as amino acids and glucose, enhance mTOR function. mTOR controls translation in part by regulating ribosomal S6 kinase (S6K) and eukaryote initiation factor 4E binding protein (4E-BP1), but may also regulate ribosomal biogenesis through nucleophosmin (NPM)[73]. Phosphorylation of the S6 protein by S6K selectively increases the ribosomal biogenesis, 4E-BP1 acts as a translation repressor by binding and inhibiting the eukaryotic translation initiation factor 4E (eIF4E).

mTOR and cancer: 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[72,73] with an average IC50 of 8.2 nM. Subsequent xenograft studies have confirmed the cytostatic properties of the mTOR inhibitors. Single agent mTOR inhibition has resulted in objective radiographic responses to a variety of mTOR inhibitors in patients with low-grade astrocytomas[74], glioblastoma multiforme[75], renal cell carcinoma[76], mantle cell lymphoma[44], breast cancer[77] and sarcomas[78]. Patients with Tuberous Sclerosis Complex (TSC) also exhibit similar deregulation of mTOR/S6K signaling. A recent clinical series has demonstrated clinical responses to single agent sirolimus in subependymal giant cell astrocytomas and a low grade glioma in 5 pediatric patients with TSC[74]. In addition, a recent North Central Cancer Treatment Group phase 2 trial of the sirolimus ester temsirolimus (CCI-779) in 65 adult patients with recurrent glioblastoma multiforme revealed radiographic improvement in 36% of the temsirolimus-treated patients, and was associated with significantly longer time to progression[75]. The most common grade 3 or higher toxicities encountered were hypercholesterolemia (13% of patients), hypertriglyceridemia (11%) and hyperglycemia (8%). Chan and colleagues treated 109 patients with relapsed, progressive advanced-stage breast cancer in a randomized trial with temsirolimus (CCI-779) at either 75 mg or 250 mg weekly by vein[77]. He found that 9% of

patients had a response (all PR); while these responses were divided equally between the low and high dose of CCI-779, toxicity was less common in the lower dose. The most common clinically important grade 3 or 4 adverse events were mucositis (9%), leucopenia (7%), hyperglycemia (7%), somnolence (6%), thrombocytopenia (5%), and depression (5%). Others have treated patients with advanced refractory renal cell carcinoma with single agent CCI-779 and reported objective response and minor response rates of 7% and 26%, respectively[76]. Grade 3 or 4 toxicities in this group of 111 patients included hyperglycemia (17%), hypophosphatemia (13%), anemia (9%), and hypertriglyceridemia (6%). MTOR inhibitors such as CCI-779, sirolimus, and everolimus have proven safe at a range of doses.

Available mTOR Inhibitors: Sirolimus and three analogs, CCI-779 (temsirolimus), everolimus (everolimus), and AP23573, have been developed for human use. Among these, only sirolimus, everolimus, and temsirolimus are currently FDA approved. Sirolimus is approved for prevention of kidney allograft rejection in adults and children at least 13 years old. In this patient population, the typical starting dose is 2 mg once daily with a single loading dose of 6 mg. Sirolimus is also approved for use in drug-eluting stents to reduce the incidence of re-stenosis following coronary artery angioplasty[79]. Temsirolimus (Wyeth) is an ester of sirolimus and is available for intravenous infusion. Temsirolimus was approved by the FDA for adults with advanced renal cell cancer on May 30, 2007 using a weekly intravenous schedule. Everolimus (Afinitor®/Votubia®) (Novartis) is an orally available hydroxyethyl derivative of sirolimus, and is currently FDA approved for advanced RCC, advanced PNET, lung neuroendocrine tumor, SEGA with TS in an individuate who is not a candidate for curative surgical resection and in Waldenström's macroglobulinemia/lymphoplasmacytic lymphoma. Additionally, in adults three Phase 3 trials are ongoing, four Phase 2 trials (alone or in combination with other agents), and in a limited number of pediatric trials. Finally AP23573 (Ariad Pharmaceuticals) is an analog of sirolimus currently in Phase 2 trials in adults. At this time, it is unclear whether one compound will have an advantage over the others in a particular tumor type.

mTOR and NF1: The *NF1* gene encodes a protein, termed neurofibromin, which functions partly as a Ras-GTPase activating protein (RasGAP). Accordingly, neurofibromin loss in tumor cells leads to Ras hyperactivation. 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. One of these downstream proteins is the mammalian target of rapamycin (mTOR) molecule. Recent 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[80,81]. In this regard, *Nf1* loss in mouse embryonic fibroblasts[81] and primary mouse astrocytes[80] 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. In addition, the increased proliferation associated with loss of neurofibromin expression in human MPNST cell lines was dramatically reduced by treatment with sirolimus. Using a genetic mouse model of NF1-deficient malignant peripheral nerve sheath tumors (MPNST) development, sirolimus completely inhibited the growth of these tumors *in vivo*[82]. In addition, sirolimus treatment of optic gliomas developing in a genetically-engineered *Nf1* mouse model resulted in attenuated mTOR signaling *in vivo*[83] as well as tumor growth *in vivo*. 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. Similarly, the mTOR inhibitor everolimus decreased growth in five MPNST cell lines, and prevented growth of subcutaneously implanted MPNST in mice[82].

Details describing studies performed by Dr. Karen Cichowski's group[81,84] in a transgenic mouse model are described below:
In this model mice carrying compound mutations in the *Nf1* and *p53* tumor suppressors on the same chromosome (referred to as NP cis animals) 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. Importantly, lesions develop as a result of somatic loss of the wild-type *Nf1* and *p53* alleles, and therefore are also genetically similar to human MPNST. This model was used to test the requirement for mTOR in tumorigenesis *in vivo* and assess the therapeutic utility of rapamycin. Animals with palpable tumors (approximately 300 mm^3) were injected I.P. with 5 mg/kg rapamycin per day. Control NP cis mice died on average in 12.2 days, and tumors grew 9.7-fold. In contrast, rapamycin potently suppressed MPNST growth, resulting in only a .04 fold increase in size, and allowed the animals to survive. In no case did a rapamycin-treated animal die, but rather animals were sacrificed for interim analysis. Thus, rapamycin has potent cytostatic effects on these highly aggressive malignancies. Inhibition of S6 phosphorylation was observed in tumor and non-tumor tissue, demonstrating that rapamcyin 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. These data demonstrate that these aggressive tumors could be completely contained by an mTOR inhibitor. mTOR inhibition through treatment with everolimus also provided benefit in a mouse xenograft model of sporadic and NF1 associated[82].

mTOR Inhibition and AKT activation: Several investigators have recently reported a negative feedback loop between mTOR and Akt in cancer cell lines[85]. Since Akt activation is associated with signaling down cell survival pathways, the effect of mTOR inhibition may be attenuated. However, in two independent NF1-associated preclinical mouse tumor models treated with sirolimus (optic glioma and malignant peripheral nerve sheath models), no evidence was found for changes in Akt activation following rapamycin treatment[81,83,84].

These findings described above identify the *NF1* tumor suppressor as a negative regulator of mTOR, and demonstrate that sirolimus blocks the growth of several *NF1*-deficient cells and tumor cell types *in vitro* and *in vivo*. The combination of cell culture and preclinical mouse modeling data provides a strong rationale for the use of sirolimus in treating human NF1-associated tumors.

However, ultimately MPNSTs become resistant to treatment with rapamycin in this model, which is associated with re-vascularization and upregulation of VEGF. Preliminary preclinical data in the transgenic NF1 MPNST mouse model demonstrate prolonged survival for mice treated with rapamycin plus sunitinib, which in part, mediates anti-tumor activity by inhibition of angiogenesis, compared to mice treated with rapamycin or sunitinib alone (unpublished data K. Cichowski). Other studies demonstrate a role of angiogenesis in progression of MPNST. Angiogenesis has also been implied in the progression of MPNST. *Ras* mutations can upregulate VEGF expression[86,87] and vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF) are also highly expressed in neurofibromas from patients with NF1 at the mRNA and protein level[88]. Furthermore, VEGF expression and tumor vascularization significantly increased in MPNSTs[89]. Use of a specific small molecular inhibitor of VEGF-receptor 2 (VEGFR2) in a mouse explant model of neurogenic sarcomas showed a reduction in tumor growth due to decreased tumor angiogenesis with subsequent reduction in tumor cell proliferation and an increase in apoptosis[89].

Based on these preclinical studies we are proposing a phase 2 clinical trial of the mTOR inhibitor everolimus (everolimus) in combination with bevacizumab in NF1 related and sporadic MPNST. The primary goal of this trial will be to evaluate the activity of everolimus in refractory MPNST, and as such this trial will serve to validate the transgenic NF1 MPNST mouse model. The intent is that this trial will serve as platform for future studies combining targeted agents or standard chemotherapy agents with mTOR inhibition based on emerging additional findings from studies in preclinical models.

2.4 Study Design

This is a two-stage phase 2 clinical trial with the objective to assess activity of the mTOR inhibitor everolimus in combination with the angiogenesis inhibitor

bevacizumab in patients with sporadic and NF1 associated MPNSTs refractory after ≥ 1 prior cytotoxic chemotherapy regimen. Novartis will provide everolimus and Genentech will provide bevacizumab for the trial.

The primary endpoint is clinical benefit rate (PR, CR or stable disease at ≥ 4 months). The diagnosis of NF1 will be based on clinical criteria.

Most MPNSTs occur in adults, but MPNSTs in adolescents and children have been described and treatment approaches are similar for adults and children with MPNST. Initially enrollment will be limited to patients ≥ 18 years old, as the phase 2 dose for the combination of everolimus and bevacizumab in children has not been defined yet. A pediatric phase 1 trial of everolimus and bevacizumab is ongoing. Everolimus will be administered at the recommended phase 2 dose. Bevacizumab will also be administered at FDA approved doses. Everolimus will be administered orally once daily as tablets at a dose of 10 mg on a continuous dosing schedule. Bevacizumab will be administered IV at a dose of 10 mg/kg/dose every 14 days. One treatment cycle will be defined as 28 days.

Patients will be regularly monitored for everolimus and bevacizumab toxicity and response. Dose modifications and interruptions will be performed as outlined in Section 6. Specific guidelines for grading and management of hypertension are described in Section 6.

Patients will receive PCP prophylaxis while on everolimus; for example trimethoprim/sulfamethoxazole as per institutional guidelines, or inhaled pentamidine.

Clinical benefit rate (success) will be defined as PR, CR, and SD at ≥ 4 months (WHO response criteria). A two-stage design will be used, and a clinical benefit rate of ≥ 25% will be defined as success ruling out a ≤ 5% clinical benefit rate. Initially 15 patients will be enrolled. If ≥ 1 of 15 patients respond, enrollment continues to 25 evaluable patients. If ≥ 4 of 25 evaluable patients respond; everolimus in combination with bevacizumab will be considered active and worthy for further exploration. Patients ≥ 18 years old who are able to swallow tablets and have adequate organ function and unresectable or metastatic MPNST which progressed after ≥ one prior cytotoxic chemotherapy regimen will be eligible. Patients will be able to remain on treatment for as long as they do not experience progressive disease or unacceptable toxicity up to a maximum of 2 years. It is expected that 15-25 patients per year will be enrolled, and enrollment is expected to complete within approximately 1-2 years depending on the activity of everolimus and bevacizumab observed during the initial stage. Response evaluations (WHO) with appropriate imaging studies (MRI/CT) will be performed before every other treatment cycle (3, 5, 7, 9, etc).. History and physical examination including vital signs will be performed after cycles 1, 2, 3 and at the time of each evaluation. Laboratory studies will include CBC with

differential, fasting glucose and lipids, comprehensive chemistry panel. Based on the preclinical studies in transgenic and subcutaneous murine MPNST models we expect to observe predominantly a cytostatic effect. Therefore response will be defined as PR, CR, and SD at ≥ 4 months using WHO response criteria. MPNSTs are very aggressive tumors. In a recently completed phase 2 trial with the EGFR inhibitor erlotinib the median progression free survival was 2 months with 19 of 20 evaluable patients demonstrating progression at the time of the first disease evaluation on treatment[36]. A subsequent phase 2 trial of sorafenib for different refractory sarcoma strata identified MPNST as the sarcoma type with the worst PFS (1.7 months) and overall survival (4.9 months) among the sarcomas evaluated[37]. We therefore believe that disease stability at ≥ 4 months indicates potential activity of the evaluated treatment.

2.5 Correlative studies

Correlative studies will not be mandatory, and patients will be able to participate in the trial without agreeing to participate in any or all of the following studies.

- To evaluate the spectrum of germline NF1 mutations in individuals with NF1 associated MPNSTs: Comprehensive NF1 mutation analysis has become available within the past few years and allows identification of an NF1 mutation in 95% of non-founder patients with > 2 neurofibromas or 1 plexiform and fulfilling the NIH clinical diagnostic criteria in a CLIA-certified laboratory[90]. This allows for meaningful analysis of phenotype and genotype correlations. Total NF1 gene deletion has been described to result in a greater risk for development of an MPNST[91]. This finding, if confirmed, would have implications for genetic counseling. In addition, obtaining the spectrum of germline NF1 mutations in individuals with NF1 and MPNST and comparing it with the spectrum of germline NF1 mutations in a cohort of adult patients without externally visible plexiform neurofibromas will enhance our knowledge. Genotyping will only be performed after genetic counseling including the potential risks of genotyping and after obtaining written informed consent.
- To explore the relationship between response to everolimus in combination with bevacizumab and presence of NF1 mutations or NF1 inactivation in MPNST tumor samples.
- To explore the response rate to everolimus in combination with bevacizumab in individuals with sporadic and NF1 associated MPNST. While NF1 inactivation has been described in sporadic and NF1 associated MPNST[92], retrospective studies describe worse survival in individuals with NF1 associated compared to sporadic MPNST[4,13], and two studies describes worse response to chemotherapy in NF1 associated versus sporadic MPNST[2,13]. This analysis will only be performed in exploratory fashion and not be powered for a statistical comparison.

- To assess preliminary correlations of radiographic response and progression with changes in pharmacodynamic parameters including S6K1 (p70 S6 kinase 1), eIF4E, eIF2 alpha, VEGF, VEGFR, Akt phosphorylation, and markers of cell metabolism in peripheral blood mononuclear cells collected prior to and serially during treatment with everolimus and bevacizumab: Markers of angiogenesis and downstream targets of mTOR will be analyzed to evaluate the effect of everolimus and bevacizumab on response and resistance. These evaluations can be performed in blood, and will thus be serially performed during treatment with everolimus at the same time as response evaluations.
- To more sensitively monitor response to everolimus and bevacizumab by using three-dimensional MRI (3D-MRI) analysis of MPNSTs compared to conventional two-dimensional MRI (2-D MRI) and one-dimensional MRI (1-D MRI) data analysis: Volumetric MRI analysis has become the standard method to evaluate the growth rate of plexiform neurofibromas on clinical trials[93,94]. This method may have utility for MPNSTs, which are typically large and have a complex shape. Volumetric MRI analysis will therefore be performed 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

3.1 Eligibility criteria

Patients must have baseline evaluations performed prior to the first dose of study drug and must meet all inclusion and exclusion criteria. Results of all baseline evaluations verifying that all inclusion and exclusion criteria have been satisfied must be reviewed by the Principal Investigator or his/her designee prior to enrollment of that 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 or legal representative prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

3.1.1 Patients ≥ 18 years old with unresectable or metastatic 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.

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

The diagnostic criteria leading to the diagnosis of NF1 and other NF1 findings will be documented on the eligibility checklist and on the form provided in Appendix V.

3.1.2 Patients must be able to swallow tablets.

3.1.3 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; See Section 11, Measurement of Effect) in two dimensions on CT or MRI scan.

3.1.4 Patients who develop a recurrence or progression (WHO criteria) of an MPNST in a previously radiated field may be enrolled if it has been at least 4 weeks since the last dose of radiation therapy. Patients who previously received radiation and develop a local or metastatic recurrence outside of the radiation field are also eligible.

3.1.5 Serum creatinine ≤ the upper limit of normal

3.1.6 Adequate unsupported hematologic function as shown by: ANC ≥ 1.0 x 10^9/L, Platelets ≥ 100,000 x 10^9/L, Hgb > 9 g/dL (transfusion of packed red blood cells allowed).

3.1.7 Adequate liver function as shown by: serum bilirubin ≤ 1.5 x ULN, ALT and AST ≤ 2.5x ULN (≤ 5x ULN in patients with liver metastases).

3.1.8 Fasting serum cholesterol ≤ 300 mg/dL OR ≤ 7.75 mmol/L AND fasting triglycerides ≤ 2.5 x ULN. NOTE: In case one or both of these thresholds are exceeded, the patient can only be included after initiation of appropriate lipid lowering medication.

3.1.9 Urine protein should be screened by spot urine analysis for Urine Protein Creatinine (UPC) ratio. For UPC ratio > 0.5, 24-hour urine protein should be obtained and the level should be < 1000 mg for patient enrollment.

Note: UPC ratio of spot urine is an estimation of the 24 urine protein excretion – a UPC ratio of 1 is roughly equivalent to a 24-hour urine protein of 1 gm. UPC ratio is calculated using one of the following formulas:

-- [urine protein]/[urine creatinine] – if both protein and creatinine are reported in mg/dL
-- [(urine protein) x0.088]/[urine creatinine] – if urine creatinine is reported in mmol/L

3.1.10 Patients must have recovered from the toxic effects of all prior therapy before entering this study. Recovery is defined as a toxicity ≤ grade 1 (CTCAE-version 4), unless otherwise specified in the inclusion and exclusion criteria. These patients must have had the last dose of chemotherapy at least 3 weeks prior to trial entry, last biologic agent within 7 days prior to trial entry, and the last dose of radiation therapy at least four weeks prior to trial entry. A minimum of 4 weeks must have passed from prior major surgical procedures, and a minimum of 7 days for core biopsies or other minor surgical procedures excluding placement of a vascular access device.

3.1.11 ECOG performance status of 0, 1, or 2 (Appendix I)

3.1.12 Cardiac function: Patients who received an anthracycline prior to enrollment on this study, must have an ejection fraction ≥ 50% documented by echocardiogram or MUGA scan.

3.1.13 Subjects of childbearing or child-fathering potential must be willing to use a medically acceptable form of birth control, which includes abstinence, while being treated on this study.

3.1.14 Informed Consent: Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial must only be done after obtaining written informed consent from all patients. This can be accomplished through an IRB-approved institutional screening protocol or the study-specific protocol. Documentation of the informed consent for screening will be maintained in the patient's research chart. Studies or procedures that were performed for clinical indications (not exclusively to determine eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

3.2 Exclusion criteria

3.2.1 Patients currently receiving anticancer therapies or who have received anticancer therapies within 3 weeks of the start of study drug (including chemotherapy, antibody based therapy, etc.).

3.2.2 At least 7 days since the completion of therapy with a biologic agent. For agents that have known adverse events occurring beyond 7 days after administration, this period must be extended beyond the time during which adverse events are known to occur.

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

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

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

3.2.6 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.

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

- Symptomatic congestive heart failure of New York Heart Association Class III or IV, unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction within 6 months of start of study drug, serious uncontrolled cardiac arrhythmia or any other clinically significant cardiac disease

- Severely impaired lung function defined as spirometry and DLCO that is 50% of the normal predicted value and/or O2 saturation that is 88% or less at rest on room air

- 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

- Active (acute or chronic) or uncontrolled severe infectious hepatitis Note: A detailed assessment of Hepatitis B/C medical history and risk factors

must be done at screening for all patients. HBV DNA and HCV RNA PCR testing are required at screening for all patients with a positive medical history based on risk factors and/or confirmation of prior HBV/HCV infection (see Appendix IV).

- 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 everolimus (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).

- Presence of serious non healing wound, active ulcer, or untreated bone fracture including tumor-related pathologic fracture.

- History of abdominal fistula, gastrointestinal perforation or intraabdominal abscess within 6 months prior to day 1.

3.2.8 Female patients who are pregnant or breast feeding, or adults of reproductive potential who are not using effective birth control methods. If barrier contraceptives are being used, these must be continued throughout the trial by both sexes. Adequate contraception must be used throughout the trial and for 8 weeks after the last dose of study drug, by both sexes. (Women of childbearing potential must have a negative urine or serum pregnancy test within 7 days prior to administration of study drugs)

3.2.9 Patients who have received prior treatment with an mTOR inhibitor (such as sirolimus, temsirolimus, everolimus) for sarcoma and patients who previously received bevacizumab.

3.2.10 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).

3.2.11 History of noncompliance to medical regimens

3.2.12 Concurrent use of anti-coagulant drugs (not including prophylactic doses), history of coagulopathy, or evidence of bleeding diathesis or coagulopathy

3.2.13 Patients unwilling or unable to comply with the protocol

3.2.14 CYP3A4 inhibitors: Patients may not be currently receiving strong inhibitors of CYP3A4, and may not have received these medications within 1 week of entry. See Appendix II.

3.2.15 Patients may not be currently receiving Seville orange, star fruit, grapefruit and their juices, and St. John's Wort affect P450 and PgP activity, and use is not allowed while on study.

3.2.16 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 2 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 Patient registration

The SARC016 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

Everolimus will be provided by Novartis. Everolimus is formulated as tablets for oral administration of 2.5 mg, 5 mg and 10 mg strength. Tablets are blister-packed under aluminum foil in units, which should be opened only at the time of administration as drug is both hygroscopic and light-sensitive.

The study drug everolimus will be self-administered. The investigator will instruct the patient to take the study drug exactly as specified in the protocol. Everolimus will be administered orally as a once daily dose of 10 mg.

Patients will be instructed to take everolimus, at the same time each day, preferably in the morning. Everolimus should be taken by the patient consistently with or without food, and remain as consistent as possible throughout the study, and in particular, during those periods when samples are being taken for pharmacodynamic analyses (see Section 2.5 and Operations Manual).

If vomiting occurs, no attempt should be made to replace the vomited dose. All dosages prescribed and dispensed to the patient and all dose changes during the study will be recorded in a patient diary (see Appendix III). Medication labels will

comply with US legal requirements and be printed in English. They will supply no information about the patient. The storage conditions for study drug will be described on the medication label.

Bevacizumab will be provided by Genentech. The bevacizumab dose will be placed into a 100 ml infusion bag. Administration will be as an intravenous infusion over 30-90 minutes, or it will be administered per the treating institution's standard policy. Bevacizumab will be administered every 14 days (on days 1 and 15), +/- 2 days. Everolimus should be given at the same time each day, and is not dependent on the time the Bevacizumab is given. Exception: hold everolimus dose until after research labs are obtained (see Appendix 10.4 - Schedule of Evaluations). The initial bevacizumab dose will be delivered as an IV infusion over 90 ± 10 minutes. If the first infusion is tolerated without infusion-associated adverse events (fevers and/or chills), the second infusion maybe delivered over 60 ± 10 minutes. If the 60 minute infusion is well tolerated, all subsequent infusions maybe delivered over 30 ± 10 minutes. If a patient experiences an infusion-associated adverse event, subsequent infusions will be given over the shortest period that was well tolerated. The patient may be pre-medicated for the next bevacizumab infusion.

5.2 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. Patients will be allowed to receive steroids for the treatment of pneumonitis.
- The CYP3A4 inhibitors and inducers and enzyme inducing anticonvulsants listed in Appendix II are prohibited on this trial. Other drugs or substances known to be inhibitors or inducers of the isoenzyme CYP3A (listed in Appendix II) should be avoided, if possible, and if unavoidable, only be used with caution, in association with everolimus as these can alter metabolism. If patient requires co-administration of moderate CYP3A4 inhibitors or PgP inhibitors (see Appendix III), reduce the dose of everolimus to half the currently used dose. Additional dose

reductions to every other day may be required to manage toxicities. If the inhibitor is discontinued the everolimus dose should be returned to the dose used prior to initiation of the moderate CYP3A4/PgP inhibitor.

- 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 during treatment with everolimus. Examples of live vaccines include intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella and TY21a typhoid vaccines.

5.2.1 Concomitant Cancer and other Therapy

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

The administration of biphosponates is permitted at investigator discretion. The risk for osteonecrosis of the jaw associated with bevacizumab and bisphosphonates should be considered (Section 8.2.8).

The medication history including complementary and alternative medications should be reviewed at the initial screening visit and at the time of subsequent visits. Patients will be instructed to consult a member of the study team prior to introduction of new medications. No other investigational therapy should be given to patients.

Oral contraceptives in preclinical and clinical data have shown everolimus to have CYP3A4 inhibitory activity rather than induction activity, induction of metabolism of contraceptive hormones by everolimus is unlikely. Consequently, administration of everolimus should not reduce the efficacy of oral contraceptives.

5.2.2 Supportive Care

- Appropriate antibiotics, blood products, antiemetics (EXCEPT systemic steroids), 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.

- **Patients must take some form of PCP prophylaxis while on everolimus**. For example: trimethoprim/sulfamethoxazole as per institutional guidelines, or inhaled pentamidine.

- Good oral hygiene and mouth care are encouraged, as mucositis is one of the toxicities of everolimus.

- 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 everolimus, 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.

Patients with positive hepatitis screen (See Appendix V):

- It is highly recommended that patients positive for HBV-DNA or HBsAg are treated prophylactically with an antiviral for 1-2 weeks prior to receiving study drug.
- The antiviral treatment should continue throughout the entire study period and for at least 4 weeks after the last dose of study drug.
- Patients on antiviral prophylaxis treatment or positive HBV antibodies should be tested for HBV-DNA according to study visit schedule.

5.3 Duration of therapy

Treatment may continue for a maximum of 2 years until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s), including significant irreversible grade 4 toxicity attributed to everolimus or bevacizumab
- Greater than 3 weeks have elapsed since the last dose of everolimus or greater than 8 weeks since the last dose of bevacizumab
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Patient is lost to follow-up
- Death

5.4 Duration of follow up

Patients will be followed until 30 days after the last dose of everolimus and bevacizumab or longer if patient removed from treatment with everolimus or bevacizumab for unacceptable adverse events will be followed until resolution or stabilization of the adverse event to ≤ grade 1 using the CTCAE v 4 as detailed in Section 7.1.2.

5.5 Criteria for removal from study

Patients will be removed from study when any of the criteria listed in Section 5.3 applies and patients have been followed as detailed in section 5.4. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dosing changes for everolimus related toxicities are described in Section 6.1, and for bevacizumab related toxicities in Section 6.2. If toxicity cannot be clearly attributed to either bevacizumab alone or to everolimus alone, the toxicity will be attributed to both agents, and modifications made accordingly. Should a patient require permanent discontinuation of either everolimus or bevacizumab, this patient can continue on study receiving the agent, which is tolerated for as long 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 everolimus or bevacizumab alone.

6.1 Interruption or discontinuation of treatment with everolimus

For patients who are unable to tolerate the protocol-specified dosing schedule, dose adjustments are permitted in order to keep the patient on study drug. If the patient misses a dose of everolimus, they may still take it up to 6 hours after the time they would normally take it. If more than 6 hours have elapsed, the patient should skip the dose for that day. The next day, they should take everolimus at the usual time. The patient should NOT take 2 doses to make up for the one that they missed. If administration of everolimus must be interrupted because of unacceptable toxicity, drug dosing will be interrupted or modified according to rules described in tables 1, 2, and 3 below: Toxicity will be assessed using the NIH-NCI Common Terminology Criteria for Adverse Events, version 4.0 (http://ctep.cancer.gov/forms/CTCAEv4.pdf).

Table 1: Everolimus dose level modification guidelines

Dose level	Dose and schedule
0 (starting dose)	10 mg daily
-1	5 mg daily
-2	2.5 mg daily

Table 2: Criteria for dose-modifications in case of suspected everolimus toxicity and re-initiation of treatment

Toxicity	Actions
Non-hematological toxicity	
≥ Grade 2 (except pneumonitis – refer to Table 3) Any grade 2 non-hematologic toxicity attributable to everolimus that persists for ≥ 7 days or is considered sufficiently medically significant or sufficiently intolerable by patients that it requires treatment interruption.	If the toxicity is tolerable to the patient, maintain the same dose. If the toxicity is intolerable to patient, interrupt everolimus until recovery to grade ≤ 1. Then reintroduce everolimus at same dose. If event returns to grade 2, then interrupt everolimus until recovery to grade ≤ 1. Then reintroduce everolimus at the next lower dose level (see Table 1 above).
Any Grade 3possibly, probably or definitely attributable to everolimus, except the following: – grade 3 nausea or vomiting of less than 3 days duration; – grade 3 transaminase elevation that returns to eligibility criteria within 7 days of study drug interruption and that does not recur upon study re-challenge with study drug; – grade 3 fasting hypercholesterolemia, hyperlipidemia, hyperglycemia; – grade 3 GGT elevation – grade 3 electrolyte abnormalities	Interrupt everolimus until recovery to grade ≤ 1. Then reintroduce everolimus at the next lower dose level (see Table 1 above).
Grade 4 possibly, probably or definitely attributable to everolimus*	Discontinue everolimus
Hematological toxicity	
ANC < 750 or Grade 3 Thrombocytopenia (platelets < 50, ≥ $25x10^9$/L)	Interrupt everolimus until recovery to platelets (≥ 100,000) and ANC of ≥ 1.0 x 10^9/L. Then resume at prior dose. If toxicity recurs after the restart of therapy, patients will be dose reduced to the next lower dose level (see Table 1above). Dose modifications for hemoglobin and grade 3 lymphopenia are not required.
Grade 3 febrile neutropenia (not life-threatening)	Interrupt everolimus until resolution of fever and neutropenia to grade ≤ 2. Hold further everolimus until the ANC ≥ 1,000/mm^3 and fever has resolved. Then resume everolimus at the next lower dose level (see Table 1 above). If febrile neutropenia recurs, discontinue everolimus.

Toxicity	Actions
Grade 4 febrile neutropenia (life-threatening)	Discontinue everolimus
Any hematological or non-hematological toxicity requiring interruption for ≥ 3 weeks	Discontinue everolimus

*Grade 3 or 4 hyperlipidemia (hypercholesterolemia and/or hypertriglyceridemia) should be managed using medical therapies (see Section 6.1.2)

6.1.1 Management of stomatitis/oral mucositis/mouth ulcers

Stomatitis/oral mucositis/mouth ulcers due to everolimus should be treated using local supportive care. Please note that investigators in earlier trials have described the oral toxicities associated with everolimus 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, everolimus will be held, and restarted with a dose reduction by 1 dose level after recovery from toxicity to grade 1 or less. If the patient is off drug for greater than or equal to 3 weeks for everolimus and greater than or equal to 8 weeks for bevacizumab, they will be permanently removed from treatment.
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 everolimus metabolism, thereby leading to higher everolimus 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 4.0.

6.1.2 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 hypercholesterolemia (> 300 - 400 mg/dL or 7.75 – 10.34 mmol/L) or Grade 2 hypertriglyceridemia (> 300 – 500 mg/dl, or > 3.42 – 5.7 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 creatine 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 [12.92 mmol/L], hold everolimus until cholesterol is 500 < mg/dl [12.92 mmol/L. If triglycerides > 700 - 1,000, and HDL is low, consider fibrate or niacin. For triglycerides > 10x ULN, hold everolimus while instituting fibrate or niacin therapy until triglycerides are < 10x ULN.

Grade 3 **hyperglycemia** has been observed in patients receiving everolimus 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 everolimus and should be monitored during everolimus therapy.

6.1.3 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 everolimus. 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 everolimus 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	Everolimus Dose Adjustment
Grade 1	CT scans with lung windows and pulmonary function testing including: spirometry, DLCO, and room air O2 saturation at rest. Repeat chest x-ray/CT scan every 2 Cycles until return to baseline.	No specific therapy is required	Administer 100% of everolimus dose.
Grade 2	CT scan with lung windows and pulmonary function testing including: spirometry, DLCO, and room air O2 saturation at rest. Repeat each subsequent Cycle until return to baseline. Consider bronchoscopy *	Symptomatic only. Prescribe corticosteroids if cough is troublesome.	Reduce everolimus dose until recovery to ≤ Grade 1. Everolimus may also be interrupted if symptoms are troublesome. After recovery to ≤ grade 1, resume treatment at the next lower dose level (see Table 1 above). Patients will be withdrawn from 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 O2 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 the next lower dose level (see Table 1 above) 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 O2 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.**

6.1.4 Management of Selected Toxicities

Infection - Patients who develop a grade 4 everolimus-related infection, or pneumocystis carinii pneumonia will be removed from protocol therapy. Patients with seasonal upper respiratory tract infections are excluded.

Allergy – Patients with grade 4 allergic reaction, including rash, will be removed from protocol treatment. Patients that come off study for allergy can be replaced.

Hypertension - Patients who develop grade 4 everolimus-related hypertension will be removed from protocol therapy.

Renal Function - If serum creatinine increases to greater than 1.5 X upper limit of normal for age, a creatinine clearance or GFR should be obtained. If the

creatinine clearance or GFR is <75% of normal for age, the patient will be removed from protocol therapy.

All interruptions or changes to study drug administration must be recorded. It will be documented whether or not each patient completed the clinical study. If for any patient either study treatment or observations were discontinued the reason will be recorded. Reasons that a patient may discontinue participation in a clinical study are listed in Section 5.3.

6.2 Interruption or discontinuation of treatment with bevacizumab

Note: There will be no dose reduction for bevacizumab. Treatment should be interrupted or discontinued for certain adverse events, as described below

Note: <u>If bevacizumab is interrupted for ANY reasons for > 8 weeks (unless otherwise specified)</u>, the patient should discontinue bevacizumab therapy on protocol.

Treatment Modification for Bevacizumab-Related Adverse Events

Event	CTCAE.v4.0 Grade	Action to be Taken
Allergic reactions, or Acute infusion reactions/ cytokine release syndrome or Anaphylaxis	Grade 1-2	Infusion of bevacizumab should be interrupted for subjects who develop dyspnea or clinically significant hypotension. For infusion-associated symptoms not specified above, infusion should be slowed to 50% or less or interrupted. Upon complete resolution of the symptoms, infusion may be continued at no more than 50% of the rate prior to the reaction and increased in 50% increments every 30 minutes if well tolerated. Infusions may be restarted at the full rate during the next cycle. Subjects who experience bronchospasm (regardless of the grade) should discontinue bevacizumab
	Grade 3-4	Discontinue bevacizumab
Thromboembolic Event (Arterial); arterial ischemia - Cardiac ischemia - Myocardial infarction - CNS ischemia (TIA, CVA) - - any peripheral or visceral arterial ischemia / thrombosis	Grade 2 (if new or worsened since bevacizumab therapy) Grade 3-4	Discontinue bevacizumab

Thromboembolic event (Venous)		
	Grade 3 OR asymptomatic Grade 4	• Hold bevacizumab treatment. If the planned duration of full-dose anticoagulation is < 2weeks, bevacizumab should be held until the full-dose anticoagulation period is over. • If the planned duration of full-dose anticoagulation is > 2 weeks, bevacizumab may be resumed during full-dose anticoagulation IF all of the criteria below are met: - The subject must not have pathological conditions that carry high risk of bleeding (e.g. tumor involving major vessels or other conditions) - The subject must not have had hemorrhagic events while on study - The subject must on stable dose of heparin or have an in-range INR (usually 2-3) on a stable dose of warfarin prior to restarting bevacizumab. • If thromboemboli worsen/recur upon resumption of study therapy, discontinue bevacizumab
	Grade 4 (symptomatic)	Discontinue bevacizumab
Hypertension (see also table below for details)	Treat with anti-hypertensive medication as needed. The goal of BP control should be consistent with general medical practice including confirming that BP is elevated across three separate measurements on three separate days and all other contributing factors have been addressed (i.e. pain, anxiety).]	
	Grade 1 (SBP 120-139 mmHg or DBP80-89 mm Hg)	Consider increased BP monitoring; start anti-hypertensive medication if appropriate
	Grade 2 asymptomatic (SBP 140-159 mmHg or DBP 90-99 mm Hg)	Begin anti-hypertensive therapy and continue bevacizumab
	· Grade 2 symptomatic (SBP 140-159 mmHg or DBP 90-99 mm Hg) − Grade 3 (≥ SBP 160 mmHg or ≥ DBP 100 mmHg	• Start or adjust anti-hypertensive medication • Hold bevacizumab until symptoms resolve AND BP < 160/90mmHg*
	Grade 4	Discontinue bevacizumab
Heart failure of LV dysfunction	Grade 3	Discontinue bevacizumab
	Grade 4	Discontinue bevacizumab
Proteinuria	[Proteinuria should be monitored by urine analysis for urine protein creatinine (UPC) ratio prior to every other dose of bevacizumab] If dipstick shows 2+ proteinuria, 24-hour urine protein should be obtained]	
	UPC ratio < 3.5 or 24-h	Continue bevacizumab

	urine protein < 2 gm	
	UPC ratio ≥ 3.5 or 24-h urine protein ≥ 2 gm	Hold bevacizumab until UPC recovers to < 3.5 or 24-h urine protein < 2 gm. Discontinue bevacizumab if urine protein does not recover to < 2 after 8 weeks or bevacizumab interruption. If proteinuria recurs discontinue bevacizumab permanently
	Grade 4 or nephrotic syndrome	Discontinue bevacizumab
Hemorrhage (CNS or pulmonary)	Grade 2-4	Discontinue bevacizumab
Hemorrhage (any other organ system)	Grade 1	• Patients receiving full-dose anticoagulation should discontinue bevacizumab. • For patients not on full-dose anticoagulation, hold bevacizumab until ALL of the following criteria are met: - the bleeding has resolved and Hb is stable - there is no bleeding diathesis that would increase the risk of therapy there is no anatomic or pathologic condition that could increase the risk of hemorrhage recurrence
	Grade 3	• Patients receiving full-dose anticoagulation should discontinue bevacizumab. • For patients not on full-dose anticoagulation, hold bevacizumab until ALL of the following criteria are met: - the bleeding has resolved and Hb is stable - there is no bleeding diathesis that would increase the risk of therapy - there is no anatomic or pathologic condition that could increase the risk of hemorrhage recurrence. • Patients who experience recurrence of grade 3 hemorrhage should discontinue study therapy.
	Grade 4	Discontinue bevacizumab permanently
Platelet count decreased	Grade 1-2 Less than lower limit of institutional normal down to 50,000	• Hold bevacizumab until resolution of platelet count to greater than or equal to 100,000 • Monitor for bleeding episodes • Weekly platelet evaluation until platelet count is greater than 100,000
	Grade 3-4 Less than 50,000	• Discontinue bevacizumab if low platelet count is attributed as possibly, probably, or definitely related to study drug • Discontinue bevacizumab until recovery to greater than or equal to institutional normal if attributed as not related or unlikely to be related
RPLS (Reversible Posterior Leukoencephalopathy syndrome **or PRES** (Posterior Reversible Encephalopathy Syndrome)		Discontinue bevacizumab permanently upon diagnosis of RPLS
Wound dehiscence requiring medical or surgical intervention		Discontinue bevacizumab
Perforation (GI, or any other organ)		Discontinue bevacizumab
Fistula (GI, pulmonary or any other organ)		Discontinue bevacizumab

Bowel obstruction	G2 requiring medical intervention	• Hold bevacizumab until complete resolution
	G3-4	• Hold bevacizumab until complete resolution • If surgery is required, patient may restart bevacizumab after full recovery from surgery, and at investigator's discretion
Other unspecified bevacizumab-related AEs (except controlled nausea/vomiting).	Grade 3	• Hold bevacizumab until symptoms resolve to < grade 1
	Grade 4	• Discontinue bevacizumab • **Upon consultation with the study chair,** resumption of bevacizumab may be considered if a patient is benefiting from therapy, and the G4 toxicity is transient, has recovered to ≤ grade 1 and unlikely to recur with retreatment.

+ Patients of childbearing age whom develop grade ≥2 toxicity based on CTCAE for irregular menstruation attributed to bevacizumab as > possible can remain on treatment with consent of the patient regarding long term risk for fertility and under the discretion of the study PI.

Specific guidelines for hypertension grading and management are provided below.

Hypertension in adults*	[Treat with anti-hypertensive medication as needed. The goal of BP control should be consistent with general medical practice] including confirming that BP is elevated across three separate measurements on three separate days and all other contributing factors have been addressed (i.e. pain, anxiety).]	
	Grade 1	Consider increased BP monitoring
	Grade 2 asymptomatic **but** diastolic BP < 100 mmHg	Begin anti-hypertensive therapy and continue bevacizumab
	-Grade 2-3 Symptomatic **OR** -Diastolic BP > 100 mmHg	Hold bevacizumab should until symptoms resolve **AND** BP < 160/90mmHg*
	Grade 4	Discontinue bevacizumab permanently

***Current CTCAE version 4 definitions used by CTEP:**

- Grade 1: Prehypertension (systolic BP 120 - 139 mm Hg or diastolic BP 80 - 89 mm Hg); intervention not indicated
- Grade 2: recurrent or persistent (≥24 hrs); symptomatic increase by > 20 mm Hg (diastolic) or to
- > 140/90 mm Hg if previously WNL; monotherapy indicated Pediatric: recurrent or persistent (≥24 hrs) BP > ULN; monotherapy
- Grade 3: more than one drug or more intensive therapy than previously used indicated
- Grade 4: life threatening consequences (e.g. malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis); urgent intervention indicated

In addition to the toxicities listed above, bevacizumab will be discontinued if it is likely related to a persistent (≥ 3 weeks) NCI-CTCAE version 4 Grade 3 or 4 adverse event or any other significant adverse event that compromises the subjects' ability to participate in the study.

Any toxicity associated or possibly associated with bevacizumab treatment should be managed according to standard medical practice.

Discontinuation of bevacizumab will have no immediate therapeutic effect. Bevacizumab has a terminal half-life of 21 days; therefore, its

discontinuation results in slow elimination over several months. There is no available antidote for bevacizumab.

If bevacizumab is held or discontinued due to toxicity, patients should continue the rest of the regimen provided that patients have no toxicities that require dose interruption or dose modification of other agents of the regimen.

Patients should be assessed clinically for toxicity prior to, during, and after each infusion. If severe bevacizumab-related toxicity, such as visceral perforation, aortic dissection, or nephritic syndrome, occurs at any time during the study, treatment with bevacizumab should be discontinued.

Bevacizumab will be withheld or discontinued for its related toxicities, but not dose-reduced.

Ovarian Failure/Irregular Menstruation:

Ovarian failure, defined as amenorrhea lasting 3 or more months with follicle-stimulating hormone (FSH) elevation (≥ 30 mIU/mL), has recently been shown to be associated with the use of bevacizumab in patients with various solid tumor receiving bevaciumab in combination with cytotoxic chemotherapies. Specifically, the incidence of ovarian failure was increased in patients receiving adjuvant bevacizumab plus mFOLFOX compared to mFOLFOX alone (34% vs. 2%). After discontinuation of bevacizumab, resumption of menses and an FSH level < 30 mIU/mL was demonstrated in 22% (7/32) of these women.

The CTCAE grading of irregular menstruation is:

- grade 1: intermittent menses with skipped menses for no more than 1-3 months,
- grade 2: intermittent menses with skipped menses for more than 4-6 months, and,
- grade 3: persistent amenorrhea for more than 6 months.

All patients enrolled on this study of childbearing potential are notified of the risk associated with bevacizumab and the fact that the long term effects of bevacizumab exposure on fertility are unknown. If patients develop > grade 2 irregular menstruation that is > possible in its attribution to bevacizumab, they are permitted to stay on drug despite this adverse event as long as the local investigator and study PI have confirmed with the patient the potential short and long term risks to fertility by continuing bevacizumab and that the patient wishes to stay on study drug despite these risks.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

For this study, all grade 3 and 4 clinical adverse events which occur after enrollment and up to 30 days after the last dose of study medication must be recorded in the appropriate case report form (CRF). Grade 1 and 2 adverse events judged to be clinically significant (for example grade ≥1 mucositis, hypertension, pneumonitis) by the treating physician should also be recorded in the appropriate CRF. Guidelines for the reporting of laboratory abnormalities are described in section 7.6.

Serious Adverse Events (SAEs), as defined below, should be reported in an expedited fashion as per section 7.8. All SAEs should be reported at the participating sites according to their institutional guidelines.

7.1 Definition of Adverse Event (AE)

An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product. Pre-existing conditions which worsen during a study are to be reported as AEs.

7.2 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. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

7.3 Drug-Adverse Event relationship

The causality relationship of study drug to the adverse event will be assessed by the investigator as either: **Definite, Probable, Possible, Unlikely, or Unrelated** . Attributions can be revised, when further information becomes available. Definitions of relationship to study medication are as follows:

Definite – The AE *is clearly related* to the study treatment.
Probable – The AE *is likely related* to the study treatment.
Possible – The AE *may be related* to the study treatment.
Unlikely – The AE *is doubtfully related* to the study treatment.
Unrelated – The AE *is clearly NOT related* to the study treatment.

Additionally, adverse events should be categorized as **expected** or **unexpected** based on the Clinical Investigator Brochure or package insert.

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

7.4 Definition of Serious Adverse Events (SAE)

A serious adverse event is any adverse event that occurs at any dose and 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)
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above.

Please note:

- 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 after study enrollment and for up to 30 days after the last dose of study medication.

7.5 Progression of Underlying Malignancy and Hospitalization

Progression of underlying malignancy is **not reported** as an adverse event if it is clearly consistent with the suspected progression of the underlying cancer.

Hospitalization due solely to the progression of underlying malignancy should NOT be reported as a serious adverse event.

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.

7.6 Guidelines for Reporting of Laboratory Abnormalities as AEs /SAEs

Any treatment-emergent abnormal laboratory result, which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis 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)

Please note: any abnormal laboratory result fulfilling the criteria for a serious adverse event (SAE) should be reported as such, in addition to being recorded as an adverse event in the CRF.

7.7 Serious Adverse Event Reporting

All serious adverse events should be submitted to SARC within 1 working day from the participating sites as detailed below. SARC is responsible to report the events within 1 working day to the trial PI, Novartis, and Genentech. All SAEs should be reported at the participating sites according to their institutional guidelines.

The site investigator must assess and record the relationship of each SAE to each specific study drug. If only limited information is initially available, follow-up reports are required and should be forwarded to SARC. SAEs must be reported on the MedWatch Form 3500A along with the completed Fax Coversheet and faxed to SARC. (See Operations Manual for SAE Fax coversheet). The original SAE Form must be kept on file at the study site.

Complications or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information.

7.8 Pregnancy

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

Any pregnancy that occurs during study participation should be reported. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

7.9 Follow-up of AEs

After the discontinuation of therapy with everolimus and/or bevacizumab, 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 CRF.

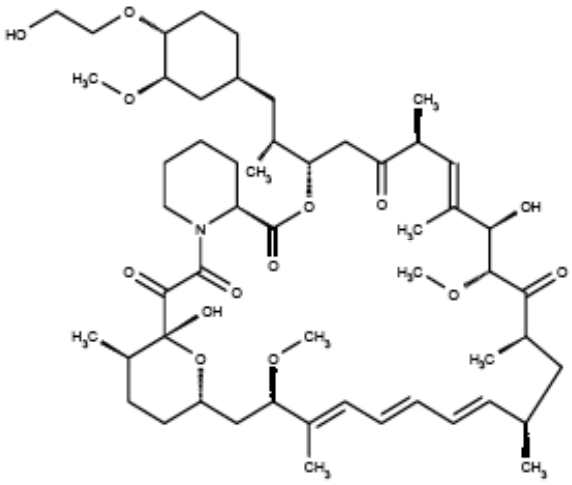
7.10 Reporting of all Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSOs) to the HRPO

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."

8. PHARMACEUTICAL INFORMATION

8.1 Everolimus (Afinitor®, Votubia®)

The structural formula and other chemical and physical properties of everolimus are summarized below:



Chemical name:
(1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-12-(1R)-2-[(1S,3R,4R)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl}-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-aza-tricyclo[30.3.1.04,9]hexatriaconta-16,24, 26,28-tetraene-2,3,10,14,20-pentaone

International non-proprietary name: Everolimus

Molecular formula: C53H83NO14

Molecular weight: 958.2

Physical form: White to faintly yellow powder. Everolimus stabilized with butylated hydroxytoluene (BHT) is amorphous, and contains 0.2% BHT as an

antioxidant.

Clinically: The drug substance, everolimus, contains 15 asymmetric carbon atoms and 4 substituted double bonds. The configuration of the asymmetric carbon atoms and the double bonds are guaranteed by the microbial origin of rapamycin, the starting material of the synthesis, and by the X-ray analysis performed on crystalline everolimus. The configuration at carbon 40 is not changed by the chemical derivatization that converts rapamycin into everolimus.

8.1.1 Preparation and administration

Tablets: For this trial: 2.5 mg, 5 mg, and 10 mg tablets will be provided.

8.1.2 Formulation, packaging and labeling

All formulations are based on a everolimus solid dispersion intermediate that was selected on the basis of the chemical stability of the active ingredient
and properties allowing for a good *in vivo* performance.

Everolimus is provided as tablets: 2.5 mg, 5 mg, and 10 mg.

Composition/ 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).

Stability: Immediate release tablets: Current stability data permit a shelf life of 36 months when stored below 30°C and protected from light and moisture.

8.1.3 Availability

Immediate release: Tablets: 2.5 mg, 5 mg and 10 mg.

8.1.4 Agent ordering

Everolimus for this trial will be provided by Novartis. Details will be provided in the Operations Manual.

8.1.5 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 orally and subjects will be asked to keep a daily diary of the time and dose of drug ingested (Appendix III).

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 TBD (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

All supplies, including partially used or empty containers will be destroyed using the site's local or institutional regulations.

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

Written documentation of destruction must contain the following:

- Identity (batch 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 Everolimus toxicities: Refer to the current Investigator's Brochure

Known Undesirable Side Effects of everolimus

Information about adverse drug reactions is mainly based on data from four randomized, double-blind, placebo-controlled phase III trials:

The most common adverse reactions (incidence ≥ 10% in at least one phase III trial and suspected to be related to treatment by the investigator) were in decreasing order: Stomatitis, rash, fatigue, diarrhea, infections, nausea, decreased appetite, anemia, dysgeusia, pneumonitis, hyperglycemia, weight decreased, pruritus, asthenia, peripheral edema, hypercholesterolemia, epistaxis, and headache.

The most common grade 3-4 adverse reactions (incidence ≥ 1/100 to <1/10 and suspected to be related to treatment by the investigator) were: stomatitis, anemia, hyperglycemia, fatigue, infections, pneumonitis, diarrhea, asthenia, thrombocytopenia, neutropenia, dyspnea, lymphopenia, proteinuria, hemorrhage, hypophosphatemia, rash, hypertension, aspartate aminotransferase (AST) increased, alanine aminotransferase (ALT) increased and pneumonia.

ADRs are listed according to MedDRA system organ class. Within each system organ class, the adverse reactions are ranked by frequency, with the most frequent reactions first. In addition, the corresponding frequency category using the following convention is also provided for each adverse reaction: very common (≥ 1/10); common (≥ 1/100 to < 1/10); uncommon (≥ 1/1,000 to < 1/100); rare (≥ 1/10,000 to < 1/1,000); very rare (< 1/10,000).

Adverse reactions reported in oncology trials

System Organ Class	Very common	Common	Uncommon	Rare
Infections and infestations	Infections[1]			
Blood and lymphatic system disorders	Anemia	Thrombocytopenia, neutropenia, leukopenia, lymphopenia	Pancytopenia	Pure red cell aplasia
Immune system disorders			Hypersensitivity	
Metabolism and nutrition disorders	Decreased appetite, hyperglycemia, hypercholesterolemia	Hypertriglyceridemia, hypophosphatemia, diabetes mellitus, hyperlipidemia, hypokalemia, dehydration		
Psychiatric disorders		Insomnia		
Nervous system disorders	Dysgeusia, headache	Ageusia		
Cardiac disorders			Congestive cardiac failure	
Vascular disorders		Hemorrhage[2] , hypertension	Deep vein thrombosis	
Respiratory, thoracic and	Pneumonitis[3], epistaxis	Cough, dyspnea	Hemoptysis, pulmonary embolism	Acute respiratory distress

mediastinal disorders				syndrome
Gastrointestinal disorders	Stomatitis[4], diarrhea, nausea	Vomiting, dry mouth, abdominal pain, oral pain, dyspepsia, dysphagia		
Skin and subcutaneous tissue disorders	Rash, pruritus	Dry skin, nail disorder, acne, erythema, hand-foot syndrome [5]		Angioedema
Musculoskeletal and connective tissue disorders		Arthralgia		
Renal and urinary disorders		Proteinuria, renal failure	Increased daytime urination, acute renal failure	
Reproductive system and breast disorders		Menstruation irregular[6]	Amenorrhea[6]	
General disorders and administration site conditions	Fatigue, asthenia, peripheral edema	Pyrexia, mucosal inflammation	Non-cardiac chest pain	Impaired wound healing
Investigations	Weight decreased	Aspartate aminotransferase increased, alanine aminotransferase increased, blood creatinine increased		
		Acne		

[1] Includes all reactions within the 'infections and infestations' system organ class including common: pneumonia and uncommon: herpes zoster, sepsis and isolated cases of opportunistic infections (e.g. aspergillosis, candidiasis, and hepatitis B)
[2] Includes different bleeding events not listed individually
[3] Includes common: pneumonitis, interstitial lung disease, lung infiltration; and rare: alveolitis, pulmonary alveolar, hemorrhage, and pulmonary toxicity
[4] Includes very common: stomatitis; common: aphthous stomatitis, mouth and tongue ulceration; uncommon: glossitis, glossodynia
[5] reported as palmar-plantar erythrodysaesthesia syndrome
[6] frequency is based upon number of women age 10 to 55 years of age in the safety pool

Clinically relevant laboratory abnormalities

In the pooled double-blind phase III safety database, the following new or worsening clinically relevant laboratory abnormalities were reported with an incidence of ≥1/10 (very common, listed in decreasing frequency).

Hematology: hemoglobin decreased, lymphocytes decreased, white blood cells decreased, platelets decreased, and neutrophils decreased (or collectively as pancytopenia);

Clinical chemistry: glucose (fasting) increased, cholesterol increased, triglycerides increased, AST increased, phosphate decreased, ALT increased, creatinine increased, and potassium decreased.

Most of the observed abnormalities (≥ 1/100) were mild (grade 1) or moderate (grade 2). Grade 3/4 hematology and chemistry abnormalities include:

Hematology: lymphocytes decreased, hemoglobin decreased (very common); neutrophils decreased, platelet count decreased, white blood cells decreased (all common).

Clinical chemistry: glucose (fasting) increased (very common); phosphate decreased, potassium decreased, AST increased, ALT increased, creatinine increased, cholesterol (total) increased, triglycerides increased (all common).

Warnings and Precautions:

- Non-infectious Pneumonitis

  Non-infectious pneumonitis is a class effect of rapamycin derivatives, including everolimus. Cases of non-infectious pneumonitis (including interstitial lung disease) have also been described in patients taking everolimus. Some of these have been severe and on rare occasions, fatal outcomes have been 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 everolimus therapy without dose alteration. If symptoms are moderate, consideration should be given to interruption of therapy until symptoms improve. The use of corticosteroids may be indicated. Everolimus may be reintroduced at 5 mg daily.

  For cases where symptoms of non-infectious pneumonitis are severe, everolimus therapy should be discontinued and the use of corticosteroids may be indicated until clinical symptoms resolve. Therapy with everolimus may be re-initiated at a reduced dose of 5 mg daily depending on the individual clinical circumstances. Pneumonitis has also been reported at a reduced dose of everolimus.

- Infections

  Everolimus has immunosuppressive properties and may predispose patients to bacterial, fungal, viral or protozoan infections, including infections with

opportunistic pathogens. Localized and systemic infections, including pneumonia, other bacterial infections, invasive fungal infections, such as aspergillosis or candidiasis and viral infections including reactivation of hepatitis B virus, have been described in patients taking everolimus[95,96]. Some of these infections have been severe (e.g. leading to respiratory or hepatic failure) and occasionally have had a fatal outcome.

Physicians and patients should be aware of the increased risk of infection with everolimus. Treat pre-existing infections prior to starting treatment with everolimus. While taking everolimus, be vigilant for symptoms and signs of infection; if a diagnosis of infection is made, institute appropriate treatment promptly and consider interruption or discontinuation of everolimus. If a diagnosis of invasive systemic fungal infection is made, discontinue everolimus and treat with appropriate antifungal therapy.

Patients with positive results of HBV-DNA and/or HBsAg at screening should begin a prophylaxis treatment for 1-2 week prior to beginning everolimus therapy. Patients should have HBV-DNA monitored frequently throughout the course of everolimus therapy for signs of hepatitis reactivation.

- If a diagnosis of invasive systemic fungal infection is made, discontinue everolimus and treat with appropriate antifungal therapy.
- Hypersensitivity reactions

  Hypersensitivity reactions manifested by symptoms including, but not limited to, anaphylaxis, dyspnea, flushing, chest pain or angioedema (e.g. swelling of the airways or tongue, with or without respiratory impairment) have been observed with everolimus.
- Oral ulceration

  Mouth ulcers, stomatitis and oral mucositis have been seen in patients treated with everolimus. In such cases topical treatments are recommended, but alcohol- or peroxide containing mouthwashes should be avoided as they may exacerbate the condition. Antifungal agents should not be used unless fungal infection has been diagnosed.
- Renal Failure Events

  Cases of renal failure (including acute renal failure), some with fatal outcome, occurred in patients treated with everolimus. Monitor renal function of patients particularly in situations where patients have additional risk factors that may further impair renal function.

Laboratory tests and monitoring

- Renal Function

  Elevations of serum creatinine and proteinuria, usually mild, have been reported in clinical trials. Monitoring of renal function, including measurement of blood

urea nitrogen (BUN), urinary protein or serum creatinine, is recommended prior to the start of everolimus therapy and periodically thereafter.

- Blood glucose

  Hyperglycemia has been reported in clinical trials. Monitoring of fasting serum glucose is recommended prior to the start of everolimus therapy and periodically thereafter. Optimal glycemic control should be achieved before starting a patient on everolimus.

- Hematologic parameters

  Decreased hemoglobin, lymphocytes, platelets and neutrophils have been reported in clinical trials. Monitoring of complete blood count is recommended prior to the start of everolimus therapy and periodically thereafter.

- Hepatic Impairment

  Everolimus exposure was increased in patients with mild (Child-Pugh A), moderate (Child-Pugh B), and severe (Child-Pugh C) hepatic impairment.

  Everolimus is not recommended for use in postmenopausal women with hormone receptor positive advanced breast cancer, or in patients with advanced neuroendocrine tumors of gastrointestinal, lung, or pancreatic origin or advanced renal cell carcinoma with severe hepatic impairment (Child-Pugh C) unless the potential benefit outweighs the risk. Everolimus is not recommended in patients with SEGA with severe hepatic impairment, (Child-Pugh class C).

- Vaccinations

  The use of live vaccines and close contact with those who have received live vaccines should be avoided during treatment with everolimus. Examples of live vaccines are: intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella, and TY21a typhoid vaccines.

8.2 Bevacizumab

Chemical name and identification:
Recombinant humanized monoclonal anti-VEGF antibody (rhuMAB VEGF)
Classification: Recombinant humanized monoclonal antibody
Mechanism of Action:
Inhibition of vascular endothelial growth factor (VEGF) resulting in inhibition of angiogenesis.
Approximate Solubility: 0.19 mg/100 mL in 0.1 N HCl, 453 mg/100 mL in Ethanol, and 2971 mg/100 mL in PEG 400.

8.2.1 How Supplied:

Bevacizumab is supplied as a clear to slightly opalescent, sterile liquid for parenteral administration. Each 400 mg (25 mg/ml – 16 mL fill) glass vial contains bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection, USP.

8.2.2 Agent Ordering:

Bevacizumab for this trial will be provided by Genentech. For agent ordering, see Operations Manual.

8.2.3 Agent Accountability

See section 8.1.5

8.2.4 Preparation

Vials contain no preservatives and are intended for single use only. Do not freeze the product. Do not shake bevacizumab during handling or preparation for clinical use.

An amount of bevacizumab needed to prepare a dose of 10 mg/kg (patient's body weight) should be withdrawn from one or more vials and diluted in a total volume of 0.9% Sodium Chloride Injection, USP, (qs.) 100 mL.

8.2.5 Storage

Bevacizumab must be stored under refrigeration at 2°–8°C (36°–46°F) upon receipt and should remain refrigerated until just prior to use. DO NOT FREEZE. DO NOT SHAKE.

Opened vials must be used within 8 hours. VIALS ARE FOR SINGLE USE ONLY. Vials used for 1 subject may not be used for any other subject. Once study drug has been added to a bag of sterile saline, the solution must be administered within 8 hours.

8.2.6 Preparation

Intact vials bear the manufacturer's expiration dating and are stable until that date if stored at 2°–8°C. Partially used vials should be discarded because the product does not contain an antimicrobial preservative. Bevacizumab should be diluted only with 0.9% Sodium Chloride Injection, USP (0.9%NS). Diluted bevacizumab solutions are stable in both

polyvinylchloride (PVC) and polyolefin containers. No significant changes were observed in protein concentration, pH, turbidity, or potency after bevacizumab dilution with 0.9% NS to concentrations of 0.9, 2.25, 3, 6.6, 7.5, and 16.5 mg/mL and storage for 24 hours in commercial PVC and polyolefin containers at 30°C. No changes were observed with respect to protein concentration, turbidity, or potency for the undiluted drug product (25 mg/mL) and after bevacizumab dilution with 0.9% NS to 1 mg/mL and 12.5 mg/mL and storage for 24 hours in non-PVC, polyolefin bags at 5°C (41°F) and 30°C (86°F).

8.2.7 Method of Administration:

Bevacizumab will be administered intravenously through a secondary IV set piggybacked above the infusion control device (pump) into a primary IV set containing 0.9% NS. When the bevacizumab drug product container is empty, 0.9% NS from the primary line should be used to flush the secondary set to complete bevacizumab delivery. 0.9% NS infusion should be continued to flush the primary IV set with a volume of fluid at least equal to the tubing priming volume, thus insuring complete drug delivery. Note that this flush is not included in the infusion times below. The initial dose should be administered over a minimum of 90 minutes. If no adverse reactions occur, the second dose should be administered over a minimum of 60 minutes. Again, if no adverse reactions occur, the third and subsequent doses should be administered over a minimum of 30 minutes. If infusion-related adverse reactions occur, subsequent infusions should be administered over the shortest period that was well tolerated.

8.2.8 Bevacizumab toxicities: Refer to current Investigator's Brochure

Bevacizumab toxicities are listed below and also described in Section 2.2.

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.3, August 1, 2013[1]

Adverse Events with Possible Relationship to Bevacizumab (rhuMAb VEGF) (CTCAE 4.0 Term) [n= 3540]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 3)***
		Blood and lymphatic system disorders - Other (renal thrombotic microangiopathy)	
	Febrile neutropenia		***Febrile neutropenia (Gr 3)***

CARDIAC DISORDERS			
		Acute coronary syndrome[2]	
	Cardiac disorders - Other (supraventricular arrhythmias)[3]		***Cardiac disorders - Other (supraventricular arrhythmias)[3] (Gr 3)***
		Heart failure	
		Left ventricular systolic dysfunction	
		Myocardial infarction[2]	
		Ventricular arrhythmia	
		Ventricular fibrillation	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 3)***
	Colitis		***Colitis (Gr 3)***
	Constipation		***Constipation (Gr 3)***
	Diarrhea		***Diarrhea (Gr 3)***
	Dyspepsia		***Dyspepsia (Gr 2)***
		Gastrointestinal fistula[4]	
	Gastrointestinal hemorrhage[5]		***Gastrointestinal hemorrhage[5] (Gr 2)***
	Gastrointestinal obstruction[6]		
		Gastrointestinal perforation[7]	
		Gastrointestinal ulcer[8]	
	Ileus		
	Mucositis oral		***Mucositis oral (Gr 3)***
	Nausea		***Nausea (Gr 3)***
	Vomiting		***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Fatigue		***Fatigue (Gr 3)***
	Infusion related reaction		***Infusion related reaction (Gr 2)***
	Non-cardiac chest pain		***Non-cardiac chest pain (Gr 3)***
	Pain		***Pain (Gr 3)***
IMMUNE SYSTEM DISORDERS			
	Allergic reaction		***Allergic reaction (Gr 2)***
		Anaphylaxis	
INFECTIONS AND INFESTATIONS			
	Infection[9]		***Infection[9] (Gr 3)***
		Infections and infestations - Other (necrotizing fasciitis)	
	Infections and infestations - Other (peri-rectal abscess)		
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
		Injury, poisoning and procedural complications – Other (anastomotic leak)[10]	
	Wound complication		***Wound complication (Gr 2)***
	Wound dehiscence		***Wound dehiscence (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 3)***

	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr 3)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 3)***
	Blood bilirubin increased		***Blood bilirubin increased (Gr 2)***
	Cardiac troponin I increased		
Neutrophil count decreased			***Neutrophil count decreased (Gr 3)***
	Platelet count decreased		***Platelet count decreased (Gr 4)***
	Weight loss		***Weight loss (Gr 3)***
	White blood cell decreased		***White blood cell decreased (Gr 3)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 3)***
	Dehydration		***Dehydration (Gr 3)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr 3)***
	Musculoskeletal and connective tissue disorder - Other (bone metaphyseal dysplasia)[11]		
	Myalgia		***Myalgia (Gr 3)***
	Osteonecrosis of jaw[12]		
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Headache		***Headache (Gr 3)***
		Intracranial hemorrhage	
		Ischemia cerebrovascular[2]	
	Peripheral sensory neuropathy[13]		
		Reversible posterior leukoencephalopathy syndrome	
	Syncope		
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
	Hematuria		***Hematuria (Gr 3)***
	Proteinuria		***Proteinuria (Gr 2)***
		Renal and urinary disorders - Other (Nephrotic Syndrome)	
		Urinary fistula	
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			
Reproductive system and breast disorders - Other (ovarian failure)[14]			
		Vaginal fistula	
	Vaginal hemorrhage		***Vaginal hemorrhage (Gr 3)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Allergic rhinitis		***Allergic rhinitis (Gr 3)***
		Bronchopleural fistula	

		Bronchopulmonary hemorrhage	
	Cough		***Cough (Gr 3)***
	Dyspnea		***Dyspnea (Gr 2)***
	Epistaxis		***Epistaxis (Gr 3)***
	Hoarseness		***Hoarseness (Gr 3)***
		Respiratory, thoracic and mediastinal disorders - Other (nasal-septal perforation)	
		Respiratory, thoracic and mediastinal disorders - Other (tracheo-esophageal fistula)	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Pruritus		***Pruritus (Gr 2)***
	Rash maculo-papular		***Rash maculo-papular (Gr 2)***
	Urticaria		***Urticaria (Gr 2)***
VASCULAR DISORDERS			
Hypertension			***Hypertension (Gr 3)***
	Thromboembolic event		***Thromboembolic event (Gr 3)***
		Vascular disorders - Other (arterial thromboembolic event)[2,15]	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]The risks of arterial thrombosis such as cardiac or CNS ischemia are increased in elderly patients and in patients with a history of diabetes.

[3]Supraventricular arrhythmias may include supraventricular tachycardia, atrial fibrillation and atrial flutter.

[4]Gastrointestinal fistula may include: Anal fistula, Colonic fistula, Duodenal fistula, Esophageal fistula, Gastric fistula, Gastrointestinal fistula, Rectal fistula, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[5]Gastrointestinal hemorrhage may include: Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Intra-abdominal hemorrhage, Oral hemorrhage, Rectal hemorrhage, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[6]Gastrointestinal obstruction may include: Colonic obstruction, Duodenal obstruction, Esophageal obstruction, Ileal obstruction, Jejunal obstruction, Rectal obstruction, Small intestinal obstruction, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[7]Gastrointestinal perforation may include: Colonic perforation, Duodenal perforation, Esophageal perforation, Gastric perforation, Jejunal perforation, Rectal perforation, Small intestinal perforation, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[8]Gastrointestinal ulcer may include: Duodenal ulcer, Esophageal ulcer, Gastric ulcer, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[9]Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

[10]Anastomotic leak may include Gastric anastomotic leak; Gastrointestinal anastomotic leak; Large intestinal anastomotic leak; Rectal anastomotic leak; Small intestinal anastomotic leak; Urostomy leak; Vaginal anastomotic leak

[11]Metaphyseal dysplasia was observed in young patients who still have active epiphyseal growth plates.

[12]Cases of osteonecrosis of the jaw (ONJ) have been reported in cancer patients in association with bevacizumab treatment, the majority of whom had received prior or concomitant treatment with i.v. bisphosphonates.

[13]Increased rate of peripheral sensory neuropathy has been observed in trials combining bevacizumab and chemotherapy compared to chemotherapy alone.

[14]Ovarian failure, defined as amenorrhea lasting 3 or more months with follicle-stimulating hormone (FSH) elevation (≥30 mIU/mL), was increased in patients receiving adjuvant bevacizumab plus mFOLFOX compared to mFOLFOX alone (34% vs. 2%). After discontinuation of bevacizumab, resumption of menses and an FSH level <30 mIU/mL was demonstrated in 22% (7/32) of these women. Long term effects of bevacizumab exposure on fertility are unknown.

[15]Arterial thromboembolic event includes visceral arterial ischemia, peripheral arterial ischemia, heart attack and stroke.

Also reported on bevacizumab (rhuMAb VEGF) trials but with the relationship to bevacizumab (rhuMAb VEGF) still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (idiopathic thrombocytopenia purpura); Bone marrow hypocellular; Disseminated intravascular coagulation; Hemolysis
CARDIAC DISORDERS - Atrioventricular block complete; Atrioventricular block first degree; Cardiac arrest; Myocarditis; Pericardial effusion; Restrictive cardiomyopathy; Right ventricular dysfunction
EAR AND LABYRINTH DISORDERS - Ear and labyrinth disorders - Other (tympanic membrane perforation); Hearing impaired; Tinnitus; Vertigo
ENDOCRINE DISORDERS - Hyperthyroidism; Hypothyroidism
EYE DISORDERS - Blurred vision; Cataract; Dry eye; Extraocular muscle paresis; Eye disorders - Other (blindness); Eye disorders - Other (conjunctival hemorrhage); Eye disorders - Other (corneal epithelial defect); Eye disorders - Other (floaters); Eye disorders - Other (ischemic CRVO); Eye disorders - Other (macular pucker); Eye disorders - Other (transient increased IOP > or =30 mm Hg); Eye disorders - Other (vitreous hemorrhage); Eye pain; Keratitis; Optic nerve disorder; Photophobia; Retinal detachment; Retinal tear; Retinopathy; Watering eyes
GASTROINTESTINAL DISORDERS - Ascites; Chelitis; Colonic stenosis; Dry mouth; Dysphagia; Enterocolitis; Esophageal pain; Esophageal stenosis; Flatulence; Gastrointestinal disorders - Other (peritonitis); Oral pain; Pancreatitis; Proctitis; Rectal mucositis; Rectal stenosis;Typhlitis
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Death NOS; Edema face; Edema limbs; Edema trunk; Facial pain; Fever; Flu like symptoms; Gait disturbance; Injection site reaction; Localized edema; Multi-organ failure; Sudden death NOS
HEPATOBILIARY DISORDERS - Cholecystitis; Gallbladder necrosis; Gallbladder obstruction; Hepatic failure; Hepatic necrosis
INFECTIONS AND INFESTATIONS - Infections and infestations - Other (aseptic meningitis)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Arterial injury; Bruising; Burn; Dermatitis radiation; Fracture
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Blood antidiuretic hormone abnormal; CD4 lymphocytes decreased; CPK increased; Carbon monoxide diffusing capacity decreased; Electrocardiogram QT corrected interval prolonged; Forced expiratory volume decreased; GGT increased; INR increased; Lipase increased; Lymphocyte count decreased; Serum amylase increased; Weight gain

METABOLISM AND NUTRITION DISORDERS - Acidosis; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hypokalemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Back pain; Bone pain; Chest wall pain; Fibrosis deep connective tissue; Generalized muscle weakness; Head soft tissue necrosis; Joint effusion; Muscle weakness lower limb; Muscle weakness upper limb; Musculoskeletal and connective tissue disorder - Other (aseptic necrotic bone); Musculoskeletal and connective tissue disorder - Other (myasthenia gravis); Musculoskeletal and connective tissue disorder - Other (polymyalgia rheumatica); Neck pain; Pain in extremity; Pelvic soft tissue necrosis; Soft tissue necrosis lower limb
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain
NERVOUS SYSTEM DISORDERS - Arachnoiditis; Ataxia; Central nervous system necrosis; Cerebrospinal fluid leakage; Cognitive disturbance; Depressed level of consciousness; Dysesthesia; Dysgeusia; Dysphasia; Encephalopathy; Extrapyramidal disorder; Facial nerve disorder; Hydrocephalus; Leukoencephalopathy; Memory impairment; Nervous system disorders - Other (increased intracranial pressure); Paresthesia; Peripheral motor neuropathy; Pyramidal tract syndrome; Seizure; Somnolence; Tremor; Vasovagal reaction
PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Depression; Insomnia; Libido decreased; Psychosis
RENAL AND URINARY DISORDERS - Bladder spasm; Chronic kidney disease; Cystitis noninfective; Renal and urinary disorders - Other (dysuria); Renal and urinary disorders - Other (ureterolithiasis); Renal hemorrhage; Urinary frequency; Urinary incontinence; Urinary retention; Urinary tract obstruction; Urinary tract pain
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Breast pain; Erectile dysfunction; Irregular menstruation; Pelvic pain; Vaginal discharge
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Atelectasis; Hypoxia; Nasal congestion; Pulmonary fibrosis; Pulmonary hypertension; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (dry nares); Respiratory, thoracic and mediastinal disorders - Other (pulmonary infarction)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Nail loss; Pain of skin; Palmar-plantar erythrodysesthesia syndrome; Photosensitivity; Purpura; Rash acneiform; Skin and subcutaneous tissue disorders - Other (diabetic foot ulcer); Skin and subcutaneous tissue disorders - Other (skin breakdown/ decubitus ulcer); Skin hyperpigmentation; Skin induration; Skin ulceration; Stevens-Johnson syndrome
VASCULAR DISORDERS - Flushing; Hot flashes; Hypotension; Lymphocele; Phlebitis; Vasculitis

Note: Bevacizumab (rhuMAb VEGF) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

9. CORRELATIVE STUDIES

9.1 Laboratory correlative studies

The following correlative studies are not mandatory for trial participation, and will only be performed in patients providing informed consent to all or any of the studies.
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 Proangiogenic factors

Proangiogenic factors will be analyzed in blood. Blood (5 mL each) samples will be collected prior to treatment with everolimus and bevacizumab, and at the time of response evaluations prior to cycles 3 and 5, and if feasible, at the time of disease progression for determination of pro-angiogenic factors: VEGF1, 2, FGF1, 2, ephrin A, angiopoietin 2, IL8, PDGFα. Details regarding the timing, collection, handling, and shipment of samples to be obtained are provided in the Operations Manual.

9.1.2 Pharmacodynamic of everolimus

Markers of the effect of everolimus S6K1 (p70s6 kinase) activity, eIF4E, eIF2α, and AKT phosphorylation will be analyzed in peripheral blood mononuclear cells obtained prior to treatment with everolimus, and at the time of response evaluations prior to cycles 3 and 5, and, if feasible, at the time of disease progression. Details regarding the timing, collection, handling, and shipment of samples to be obtained are provided in The Operations Manual.

9.1.3 Germline NF1

One blood sample will be obtained prior to treatment with everolimus and bevacizumab to determine germline NF1 mutations in patients with a clinical diagnosis of NF1. This analysis will be performed in a CLIA approved laboratory. Details regarding informed consent, sample collection and shipment are provided in the Operations Manual.

9.1.4 MRI 3-dimentional analysis

To evaluate the utility of 3-dimentional MRI analysis for MPNST, MRIs obtained for disease evaluation at baseline, and as part of the response evaluation will be analyzed using 3D MRI in addition to 1D, and 2-D analysis. Details regarding the handling and shipment of MRI studies 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 7 days prior to enrollment of this trial unless otherwise specified:

- Informed consent

- History/demographics
- Physical exam
- Vital signs:
  Height (first visit), pulse, blood pressure, respiration rate, oxygen saturation by pulse oximeter, temperature and weight. Blood pressure, pulse and respiration rate should be measured on patients after at least 3 minutes in the sitting position.
- Performance Status (see Appendix I)
- Documentation of clinical findings of NF1 (Appendix V)
- 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 testing and lipid testing**
- Urinalysis:
  Standard urinalysis dipstick assessment (pH, protein, glucose, blood, ketones, and leukocytes) should be performed. If the results demonstrate any potentially relevant abnormalities, the dipstick assessment must be supplemented with laboratory quantification. If the site does not utilize dipsticks for assessment, the urine will be sent to the lab to be processed according to the institution's processes.
  Urine protein creatinine (UPC) ratio:
  For UPC ratio > 0.5 a 24-hour urine should be obtained and the level should be less than 1000 mg for patient enrollment.
  Note: UPC ratio of spot urine is an estimation of the 24 urine protein excretion – a UPC ratio of 1 is roughly equivalent to a 24-hour urine protein of 1 gm. UPC ratio is calculated using one of the following formulas:
  - [urine protein]/[urine creatinine] – if both protein and creatinine are reported in mg/dL
  - [(urine protein) x0.088]/[urine creatinine] – if urine creatinine is reported in mmol/L
- Serum or urine pregnancy:
  Standard pregnancy tests will be given to all females of childbearing age within **7 days** prior to starting treatment medication.
- Echocardiogram or MUGA scan for patients who received an anthracyline prior to evaluation for this study.
- Disease evaluation using appropriate test (CT and/or MRI) must be performed within 3 weeks of trial entry
- A detailed assessment of hepatitis B/C medical history and risk factors must be done for all patients at screening.

Testing for hepatitis B viral load and serologic markers: HBV-DNA, HBsAg, HBs Ab, and HBc Ab and HCV RNA PCR are required at screening for all patients in the following risk categories:

- All patients who currently live in (or have lived in) Asia, Africa, Central and South America, Eastern Europe, Spain, Portugal, and Greece.
- Patients with any of the following risk factors:
  - known or suspected past hepatitis B infection,
  - blood transfusion(s) prior to 1990,
  - current or prior IV drug users,
  - current or prior dialysis,
  - household contact with hepatitis B infected patient(s),
  - current or prior high-risk sexual activity,
  - body piercing or tattoos,
  - mother known to have hepatitis B
  - history suggestive of hepatitis B infection, e.g., dark urine, jaundice, right upper quadrant pain.

Patients with positive hepatitis B and/or hepatitis C test results must begin a course of antivirals 1-2 weeks prior to the first dose of everolimus (as outlined in Appendix IV).

10.2 On study evaluations

The following procedures will be performed during the treatment period within 8 days of the stated time point:

- History and physical:
  After cycles 1, 2, 3, and at the time of each evaluation (monthly)
- Vital Signs:
  After cycles 1, 2, 3, and at the time of each evaluation (monthly): Height (first visit), pulse, blood pressure, respiration rate, **oxygen saturation by pulse oximeter**, temperature and weight. Blood pressure, pulse and respiration rate should be measured on patients after at least 3 minutes in the sitting position.
- Performance Status (see Appendix I):
  After cycles 1, 2, 3, and at the time of each evaluation (monthly)
- Hematology:
  After cycles 1, 2, 3, and at the time of each evaluation (monthly): Hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential, PT, PTT.
- Serum Chemistry:
  After cycles 1, 2, 3, and at the time of each evaluation (monthly): 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).

- Urinalysis:
  After cycles 1, 2, 3, and at the time of each evaluation (monthly):
  Standard urinalysis dipstick assessment (pH, protein, glucose, blood, ketones, and leukocytes) should be performed. If the results demonstrate any potentially relevant abnormalities, the dipstick assessment must be supplemented with laboratory quantification. If the site does not utilize dipsticks for assessment, the urine will be sent to the lab to be processed according to the institution's processes.
  Urine protein creatinine (UPC) ratio (see Section 10.1).
- Echocardiogram or MUGA scan for patients who received an anthracyline prior to evaluation for this study, to be performed prior to every other treatment cycle (before cycles 3, 5, 7, 9, etc.).
- 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.
- Patients on antiviral prophylaxis treatment or positive HBV antibodies should be tested for HBV-DNA prior to every cycle to monitor for re-activation. Patients with positive HCV RNA-PCR results at screening and/or a history of past infection (even if treated and considered 'cured') should have HCV RNA-PCR testing performed prior to every cycle to monitor for reactivation.
- Administration of study drug:
  An everolimus diary will be kept by the patient and/or proxy to document each dose of drug taken. The diary will be reviewed after completion of cycles 1, 2, and 3, and at the time of each evaluation (monthly).
- Recording of adverse events*:*
  After each treatment cycle (monthly)

Correlative studies:

- Blood sample for proangiogenic factors:
  Prior to treatment with everolimus and bevacizumab, and at the time of response evaluations prior to cycles 3 and 5 (see Operations Manual).
- Blood samples as marker for everolimus effect:
  Prior to treatment with everolimus and at the time of response evaluations prior to cycles 3 and 5 (see Operations Manual).

- Blood sample for determination of NF1 mutation in individuals with NF1 associated MPNSTs: One time point prior to treatment with everolimus and bevacizumab. (see Operations Manual).
- 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 in addition to standard 2-dimensional response evaluations (see Operations Manual).

10.3 End of treatment/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. Blood pressure, pulse and respiration rate should be measured on patients after at least 3 minutes in the sitting position.
- 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).
- Urinalysis:
  Standard urinalysis dipstick assessment (pH, protein, glucose, blood, ketones, and leukocytes) should be performed. If the results demonstrate any potentially relevant abnormalities, the dipstick assessment must be supplemented with laboratory quantification. If the site does not utilize dipsticks for assessment, the urine will be sent to the lab to be processed according to the institution's processes. UPC ratio (see Section 10.1)
- Patients on antiviral prophylaxis treatment or positive HBV antibodies should be tested for HBV-DNA. Patients with positive HCV RNA-PCR results at screening and/or a history of past infection (even if treated and considered ‘cured’) should have HCV RNA-PCR testing performed.
- Echocardiogram or MUGA scan for patients who received an anthracyline prior to evaluation for this study.
- Disease evaluation using appropriate test (CT and/or MRI).

Correlative studies:

- Blood sample for proangiogenic factors:
  If feasible, at the time of disease progression (see Operations Manual).

- Blood samples as marker for everolimus effect:
  If feasible, at the time of disease progression (see Operations Manual).

- 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 in addition to standard 2-dimensional response evaluations (see Operations Manual).

10.4 Schedule of Evaluations

	Treatment Period																								Off Study
Cycle Number		1(a)				2(a)				3(a)				4(a)				5(a)				6 (a) (b)				
Study Week	Screen (c)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Everolimus – To be orally administered by patient		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Bevacizumab		X		X		X		X		X		X		X		X		X		X		X		X		
Informed Consent/Assent	X																									
Demographics	X																									
History and Physical Exam with Vital signs (d)	X					X				X				X				X				X				X
Documentation of NF1 Findings (f)	X																									
Everolimus Diary Review						X				X				X				X				X				X
Performance Status (e)	X					X				X				X				X				X				X
CBC w/diff., Platelets PT, PTT (g)	X					X				X				X				X				X				X
Serum Chemistry (h)	X					X				X				X				X				X				X
Urinalysis, UPC ratio (i)	X					X				X				X				X				X				X
Urine or Serum Pregnancy Test (j)	X																									
Hepatitis labs (k)	X					X				X				X				X				X				X
Disease evaluation with appropriate radiographic studies (l)	X									X								X								X
Echocardiogram or MUGA scan (m)	X									X								X								X
Pharmacodynamic Studies:	X									X								X								X(n)

proangiogenic factors, markers of everolimus effect																									
Genotyping for NF1 MPNST	**X**																								
MRI for 3-D Analysis	**X**									**X**								**X**							**X(n)**
Adverse Events		**Continuously throughout the study**																							**X**
Concomitant Medication	**Continuously throughout the study**																								**X**

a Evaluations obtained prior to the next cycle. Evaluations can be performed within 8 days of the requested time.

b Patients who continue on study should have evaluations as shown in cycle 6 performed prior to each cycle 8, 10, 12, 14 etc.

c All studies to determine eligibility must be performed within 1 week prior to enrollment unless otherwise indicated (See section 10.1).

d Vital signs: Height (first visit only), pulse, blood pressure, respiratory rate, oxygen saturation by pulse oximeter, temperature and weight. Blood pressure, pulse and respiration rate should be measured on patients after at least 3 minutes in the sitting position.

e See Appendix I for performance status criteria.

f See Appendix V for documentation of findings of NF1.

g Hematology: hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential, PT, PTT.

h 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 testing and lipid testing.

i Urinalysis: Standard urinalysis dipstick assessment (pH, protein, glucose, blood, ketones, and leukocytes) should be performed. If the results demonstrate any potentially relevant abnormalities, the dipstick assessment must be supplemented with laboratory quantification. If the site does not utilize dipsticks for assessment, the urine will be sent to the lab to be processed according to the institution's processes.

j Standard pregnancy tests will be given to all females of childbearing age ≤ **7 days** prior to starting treatment medication, and subsequently as clinically indicated and following institutional guidelines.

k For patients at risk prior to enrollment, and for patients on antiviral hepatitis prophylaxis prior to each subsequent cycle. See Appendix IV

l Performed ≤ 3 weeks prior to trial entry. Disease evaluation using appropriate test (CT and/or MRI) for volumetric analysis must be performed within 3 weeks of trial entry and 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.

m Required only for patients who received an anthracycline prior to enrollment on this study, performed ≤ 3 weeks prior to trial entry.

n At the time of disease progression (if feasible).

11. MEASUREMENT OF EFFECT

11.1 Antitumor effect – solid tumor

11.1.1 Definitions

Evaluable for toxicity: 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 everolimus and bevacizumab 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 $\geq$ 20 mm 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 < 20 mm 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 everolimus and bevacizumab 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 bevacizumab doses and $\geq$ 70% of the required everolimus 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. 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 5 mm or less in slice thickness contiguously. 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 response criteria 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, 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 $\geq$ 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, $\geq$ 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.

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, 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 Chief Executive Officer, and a biostatistician.

Safety oversight for this trial is also supported by the SARC Clinical Research Committee which is made up of senior sarcoma investigators and the SARC President and Chief Executive Officer. This committee also convenes monthly. 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 the Genentech and Novartis. 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 for 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

There will be approximately 10 SARC and NF Consortium sites collaborating to accrue patients to this study. We anticipate that accrual will take approximately 2 years.

This trial will be 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. 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: sarc@sarctrials.org

As this trial receives funding by the US Army, 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. SARC will submit these documents to the USAMRMC Office of Research Protections (ORP), Human Research Protections Office (HRPO).

Major modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the USAMRMC ORP HRPO for approval prior to implementation. The USAMRMC ORP HRPO defines a substantive modification as a change in Principal Investigator, change or addition of an institution, elimination or alteration of the consent process, change to the study population that has regulatory implications (e.g. adding children, adding active duty population, etc.), significant change in study design (i.e. would prompt additional scientific review) or a change that could potentially increase risks to subjects. 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 website 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 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 website.

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, enrollment will be limited to patients ≥ 18 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 everolimus and bevacizumab, the FDA approved agents used to treat the refractory MPNST. The primary objective of this phase 2 trial is to 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 all 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 greater than minimal risk protocol 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 Novartis, and Genentech.

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 sponsored and 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 Primary objectives

The primary endpoint of the study is the clinical benefit rate (complete response, partial response, and stable disease at ≥ 4 months) of patients with progressive high-grade, unresectable or metastatic MPNSTs treated on study.

A recently completed trial of the EGFR inhibitor erlotinib for individuals with refractory MPNST demonstrated a median time to progression of 48 days and progressive disease at the time of the first response evaluation after completion of 2 treatment cycles in 19/20 evaluable patients[36,97]. Three other smaller studies had showed similar results (see section 2.1). We therefore believe that stable disease at ≥ 4 months on treatment with everolimus alone and in combination with bevacizumab can be considered as potentially beneficial and worthy of further exploration. An evaluable patient will be classified a success for the primary endpoint if the patient achieves a PR, CR or stable disease at ≥ 4 months.

The target success rate will be 25%, and a success rate < 5% will be considered uninteresting. Using a two-stage Simon Minmax phase II design[98] the first stage will require 15 patients, with no further accrual if no successes are observed within the first 15 patients. If at least one success is observed accrual will continue until a total of 25 evaluable patients have been enrolled. If at least four successes are observed in the 25 patients everolimus with addition of bevacizumab will be considered active in that it would be consistent with a 25% success rate. Assuming the number of successes is binomially distributed, this

study has an alpha of 5% and a power of 90% for detecting a true success probability of at least 25% versus the null hypothesis success rate of 5% or less. Patients will be able to remain on treatment for as long as they do not experience progressive disease or unacceptable toxicity. It is expected that 15-25 patients per year will be enrolled, thus enrollment is expected to complete within one to two years depending on the initial number of responses observed.

All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be evaluable for response.

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

Toxicity data will be presented by severity. The incidence of toxicities will be estimated using the binomial proportion and its 95% confidence interval. The incidence of infection related deaths will be estimated using the binomial proportion and its 95% confidence interval.

13.2 Secondary objectives

Summary statistics will be used to describe the study population (such as ranges, medians of ages, gender, baseline performance characteristics).

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 scatterplots and correlation coefficients.

Pharmacodynamic parameters including p70 S6 kinase 1 activity in PBMCs and proangiogenic factors in blood samples will be measured pre-treatment and at time of response evaluations during subsequent courses. Changes in these parameters will be correlated with radiographic response using the logistic regression model.

Comprehensive NF1 mutation analysis and detailed phenotyping including quantification of tumor related and non-tumor related NF1 manifestations will be performed. Genotyping will be performed in a CLIA approved laboratory. Results from genotyping in our cohort of patients with MPNST will be compared to the spectrum of >3000 mutations as identified in a large cohort of unrelated patients.

The mutations will be subdivided in 3 main classes:

1. Nonsense, out-of frame splice mutations, frameshift mutations: these mutations are all generally accepted to lead to a premature stop codon. This results in the formation of unstable transcripts that will become degraded by the nonsense-mediated RNA decay. Hence, these types of mutations can be considered as

mutations leading to haplo-insufficiency.

2. Missense mutations and in-frame splice mutations *may* fall under a different category of mutations that may either present *hypomorphic* alleles due to some residual neurofibromin function or present *gain-of-function* alleles due to the activation or specific inhibition of a particular domain. These mutations will be subdivided according to the known and putative (*in silico* predicted) protein domains they are residing in. A distinction will be made between missense mutations residing in at least 6 regions: all exons prior to the Cystein-Serine-Rich-Domain (CSRD), the CSRD, the Tubulin-binding domain, the Gap-Related-Domain, the Sec-14 domain, and the Syndecan binding region.

3. Finally, mutations deleting the *NF1* gene as well as all genes residing in the most common 1.5 Mb deletion versus only a portion of the flanking genes. Using a Multiple Ligation Probe Assay (MLPA) and custom designed Agilent oligo CGH-array the extent of the deletion in all subjects will be established and compared with the severity of the phenotype.

It will be tested whether an association between the type of *NF1* mutation and NF1 manifestation and complications related to NF1 exists. The common clinical abnormalities will be coded as: Lisch nodules (present/ absent), number of CAL macules (0, 1–5, 6-100, > 100), axillary freckling (present/absent; unilateral/bilateral), number of cutaneous and dermal neurofibromas (0, 2-6, > 6-100, 101–500, > 500), plexiform neurofibromas (0, 1 or > 2 plexiform neurofibromas residing in different parts of the body), optic glioma (present/absent; symptomatic/asymptomatic), internal or spinal neurofibromas (present/ absent and numerically); skeletal abnormalities including tibial dysplasia, pseudarthrosis, sphenoid wing dysplasia, dysplastic vertebrae, osseous cysts and scoliosis (present/absent); other neoplasms including brainstem glioma, pheochromocytoma, rhabdomyosarcoma, malignant peripheral nerve sheath tumor, JMML, hypothalamic glioma (present/absent); cardiovascular disease (present/absent); developmental delay and learning disabilities (present/absent); NF-Noonan phenotype (present/absent); Watson syndrome phenotype (present/absent).

Logistic regression analysis will be used to calculate significance, relative risks (RR) and 95% confidence intervals (CI) for binary outcomes. Linear regression analysis will be used for continuous outcomes. Covariate information such as gender, type of constitutional NF1 mutation and age at examination (as a continuous variable) to name a few will be considered for inclusion in the models. The mutation covariate is coded as several binary variables (indicators of missense, in-frame and out-of frame splicing, and non-sense mutations). Age at examination will be included as a covariate because the penetrance of many NF1 disease features changes with age. Adjustments for multiple testing will be conducted.

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 (WHO)[94]. Similar to plexiform neurofibromas, MPNSTs have a complex shape (non spherical), and RECIST (1-dimensional)[99] or WHO (2-dimensional)[100] 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.

REFERENCES:

1. Ferner RE, Gutmann DH: International consensus statement on malignant peripheral nerve sheath tumors in neurofibromatosis. Cancer Res 62:1573-7, 2002
2. Ferrari A, Miceli R, Rey A, Oberlin O, Orbach D, Brennan B, Mariani L, Carli M, Bisogno G, Cecchetto G, De Salvo GL, Casanova M, Vannoesel MM, Kelsey A, Stevens MC, Devidas M, Pappo AS, Spunt SL: Non-metastatic unresected paediatric non-rhabdomyosarcoma soft tissue sarcomas: results of a pooled analysis from United States and European groups. Eur J Cancer 47:724-31
3. Cichowski K, Jacks T: NF1 tumor suppressor gene function: narrowing the GAP. Cell 104:593-604, 2001
4. Evans DG, Baser ME, McGaughran J, Sharif S, Howard E, Moran A: Malignant peripheral nerve sheath tumours in neurofibromatosis 1. J Med Genet 39:311-4, 2002
5. Korf BR: Plexiform neurofibromas. Am J Med Genet 89:31-7, 1999
6. Ducatman BS, Scheithauer BW, Piepgras DG, Reiman HM, Ilstrup DM: Malignant peripheral nerve sheath tumors. A clinicopathologic study of 120 cases. Cancer 57:2006-21, 1986
7. King AA, Debaun MR, Riccardi VM, Gutmann DH: Malignant peripheral nerve sheath tumors in neurofibromatosis 1. Am J Med Genet 93:388-92, 2000
8. Zou C, Smith KD, Liu J, Lahat G, Myers S, Wang WL, Zhang W, McCutcheon IE, Slopis JM, Lazar AJ, Pollock RE, Lev D: Clinical, pathological, and molecular variables predictive of malignant peripheral nerve sheath tumor outcome. Ann Surg 249:1014-22, 2009
9. Mautner VF, Friedrich RE, von Deimling A, Hagel C, Korf B, Knofel MT, Wenzel R, Funsterer C: Malignant peripheral nerve sheath tumours in neurofibromatosis type 1: MRI supports the diagnosis of malignant plexiform neurofibroma. Neuroradiology 45:618-25, 2003
10. Ferner RE, Golding JF, Smith M, Calonje E, Jan W, Sanjayanathan V, O'Doherty M: [18F]2-fluoro-2-deoxy-D-glucose positron emission tomography (FDG PET) as a diagnostic tool for neurofibromatosis 1 (NF1) associated malignant peripheral nerve sheath tumours (MPNSTs): a long-term clinical study. Ann Oncol 19:390-4, 2008
11. Scaife CL, Pisters PW: Combined-modality treatment of localized soft tissue sarcomas of the extremities. Surg Oncol Clin N Am 12:355-68, 2003
12. Kattan MW, Leung DH, Brennan MF: Postoperative nomogram for 12-year sarcoma-specific death. J Clin Oncol 20:791-6, 2002
13. Carli M, Ferrari A, Mattke A, Zanetti I, Casanova M, Bisogno G, Cecchetto G, Alaggio R, De Sio L, Koscielniak E, Sotti G, Treuner J: Pediatric malignant peripheral nerve sheath tumor: the Italian and German soft tissue sarcoma cooperative group. J Clin Oncol 23:8422-30, 2005
14. Sordillo PP, Helson L, Hajdu SI, Magill GB, Kosloff C, Golbey RB, Beattie EJ: Malignant schwannoma--clinical characteristics, survival, and response to therapy. Cancer 47:2503-9, 1981

15. Cashen DV, Parisien RC, Raskin K, Hornicek FJ, Gebhardt MC, Mankin HJ: Survival data for patients with malignant schwannoma. Clin Orthop Relat Res:69-73, 2004

16. Watson MA, Perry A, Tihan T, Prayson RA, Guha A, Bridge J, Ferner R, Gutmann DH: Gene expression profiling reveals unique molecular subtypes of Neurofibromatosis Type I-associated and sporadic malignant peripheral nerve sheath tumors. Brain Pathol 14:297-303, 2004

17. Basu TN, Gutmann DH, Fletcher JA, Glover TW, Collins FS, Downward J: Aberrant regulation of ras proteins in malignant tumour cells from type 1 neurofibromatosis patients. Nature 356:713-5, 1992

18. DeClue JE, Papageorge AG, Fletcher JA, Diehl SR, Ratner N, Vass WC, Lowy DR: Abnormal regulation of mammalian p21ras contributes to malignant tumor growth in von Recklinghausen (type 1) neurofibromatosis. Cell 69:265-73, 1992

19. Guha A, Lau N, Huvar I, Gutmann D, Provias J, Pawson T, Boss G: Ras-GTP levels are elevated in human NF1 peripheral nerve tumors. Oncogene 12:507-13, 1996

20. Kluwe L, Friedrich R, Mautner VF: Loss of NF1 allele in Schwann cells but not in fibroblasts derived from an NF1-associated neurofibroma. Genes Chromosomes Cancer 24:283-5, 1999

21. Kluwe L, Friedrich RE, Mautner VF: Allelic loss of the NF1 gene in NF1-associated plexiform neurofibromas. Cancer Genet Cytogenet 113:65-9, 1999

22. Perry A, Kunz SN, Fuller CE, Banerjee R, Marley EF, Liapis H, Watson MA, Gutmann DH: Differential NF1, p16, and EGFR patterns by interphase cytogenetics (FISH) in malignant peripheral nerve sheath tumor (MPNST) and morphologically similar spindle cell neoplasms. J Neuropathol Exp Neurol 61:702-9, 2002

23. Skotheim RI, Kallioniemi A, Bjerkhagen B, Mertens F, Brekke HR, Monni O, Mousses S, Mandahl N, Soeter G, Nesland JM, Smeland S, Kallioniemi OP, Lothe RA: Topoisomerase-II alpha is upregulated in malignant peripheral nerve sheath tumors and associated with clinical outcome. J Clin Oncol 21:4586-91, 2003

24. Abbas JS, Holyoke ED, Moore R, Karakousis CP: The surgical treatment and outcome of soft-tissue sarcoma. Arch Surg 116:765-9, 1981

25. Gupta G, Mammis A, Maniker A: Malignant peripheral nerve sheath tumors. Neurosurg Clin N Am 19:533-43, v, 2008

26. Pisters PW, Leung DH, Woodruff J, Shi W, Brennan MF: Analysis of prognostic factors in 1,041 patients with localized soft tissue sarcomas of the extremities. J Clin Oncol 14:1679-89, 1996

27. Yang JC, Chang AE, Baker AR, Sindelar WF, Danforth DN, Topalian SL, DeLaney T, Glatstein E, Steinberg SM, Merino MJ, Rosenberg SA: Randomized prospective study of the benefit of adjuvant radiation therapy in the treatment of soft tissue sarcomas of the extremity. J Clin Oncol 16:197-203, 1998

28. Wong WW, Hirose T, Scheithauer BW, Schild SE, Gunderson LL: Malignant peripheral nerve sheath tumor: analysis of treatment outcome. Int J Radiat Oncol Biol Phys 42:351-60, 1998

29. Verma S, Bramwell V: Dose-intensive chemotherapy in advanced adult soft tissue sarcoma. Expert Rev Anticancer Ther 2:201-15, 2002

30. Antman KH, Montella D, Rosenbaum C, Schwen M: Phase II trial of ifosfamide with mesna in previously treated metastatic sarcoma. Cancer Treat Rep 69:499-504, 1985

31. Edmonson JH, Buckner JC, Long HJ, Loprinzi CL, Schaid DJ: Phase II study of ifosfamide-etoposide-mesna in adults with advanced nonosseous sarcomas. J Natl Cancer Inst 81:863-6, 1989

32. Raney B, Schnaufer L, Ziegler M, Chatten J, Littman P, Jarrett P: Treatment of children with neurogenic sarcoma. Experience at the Children's Hospital of Philadelphia, 1958-1984. Cancer 59:1-5, 1987

33. Valdes OS, Maurer HM: Combination therapy with vincristine sulfate (NSC-67574) and cyclophosphamide (NSC-26271) for generalized malignant schwannoma--a case report. Cancer Chemother Rep 54:65-8, 1970

34. Santoro A, Tursz T, Mouridsen H, Verweij J, Steward W, Somers R, Buesa J, Casali P, Spooner D, Rankin E, et al.: Doxorubicin versus CYVADIC versus doxorubicin plus ifosfamide in first-line treatment of advanced soft tissue sarcomas: a randomized study of the European Organization for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group. J Clin Oncol 13:1537-45, 1995

35. Widemann BC: Current status of sporadic and neurofibromatosis type 1-associated malignant peripheral nerve sheath tumors. Curr Oncol Rep 11:322-8, 2009

36. Albritton K, Rankin C, Coffin C, Ratner N, Budd T, Schuetze S, Randall R, DeClue J, Borden E: Phase II trial of erlotinib in metastatic or unresectable malignant peripheral nerve sheath tumor (MPNST), ASCO, 2006

37. Maki RG, D'Adamo DR, Keohan ML, Saulle M, Schuetze SM, Undevia SD, Livingston MB, Cooney MM, Hensley ML, Mita MM, Takimoto CH, Kraft AS, Elias AD, Brockstein B, Blachere NE, Edgar MA, Schwartz LH, Qin LX, Antonescu CR, Schwartz GK: Phase II study of sorafenib in patients with metastatic or recurrent sarcomas. J Clin Oncol 27:3133-40, 2009

38. Schuetze S, Wathen S, Choy E, Samuels B, Ganjoo K, Staddon A, von Mehren M, Chow W, Trent J, Baker L: Results of a Sarcoma Alliance for Research through Collaboration (SARC) phase II trial of dasatinib in previously treated, high-grade, advanced sarcoma., ASCO. J Clin Oncol 28:15s, 2010 (suppl; abstr 10009) 2010

39. Chugh R, Wathen JK, Maki RG, Benjamin RS, Patel SR, Meyers PA, Priebat DA, Reinke DK, Thomas DG, Keohan ML, Samuels BL, Baker LH: Phase II multicenter trial of imatinib in 10 histologic subtypes of sarcoma using a bayesian hierarchical statistical model. J Clin Oncol 27:3148-53, 2009

40. Boulay A, Lane HA: The mammalian target of rapamycin kinase and tumor growth inhibition. Recent Results Cancer Res 172:99-124, 2007

41. Bjornsti MA, Houghton PJ: The TOR pathway: a target for cancer therapy. Nat Rev Cancer 4:335-48, 2004

42. Manning BD, Cantley LC: AKT/PKB signaling: navigating downstream. Cell 129:1261-74, 2007

43. Wullschleger S, Loewith R, Hall MN: TOR signaling in growth and metabolism. Cell 124:471-84, 2006

44. Witzig TE, Geyer SM, Ghobrial I, Inwards DJ, Fonseca R, Kurtin P, Ansell SM, Luyun R, Flynn PJ, Morton RF, Dakhil SR, Gross H, Kaufmann SH: Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. J Clin Oncol 23:5347-56, 2005

45. Zitzmann K, De Toni EN, Brand S, Goke B, Meinecke J, Spottl G, Meyer HH, Auernhammer CJ: The novel mTOR inhibitor RAD001 (everolimus) induces antiproliferative effects in human pancreatic neuroendocrine tumor cells. Neuroendocrinology 85:54-60, 2007

46. Zeng Z, Sarbassov dos D, Samudio IJ, Yee KW, Munsell MF, Ellen Jackson C, Giles FJ, Sabatini DM, Andreeff M, Konopleva M: Rapamycin derivatives reduce mTORC2 signaling and inhibit AKT activation in AML. Blood 109:3509-12, 2007

47. Ikezoe T, Nishioka C, Bandobashi K, Yang Y, Kuwayama Y, Adachi Y, Takeuchi T, Koeffler HP, Taguchi H: Longitudinal inhibition of PI3K/Akt/mTOR signaling by LY294002 and rapamycin induces growth arrest of adult T-cell leukemia cells. Leuk Res 31:673-82, 2007

48. Wanner K, Hipp S, Oelsner M, Ringshausen I, Bogner C, Peschel C, Decker T: Mammalian target of rapamycin inhibition induces cell cycle arrest in diffuse large B cell lymphoma (DLBCL) cells and sensitises DLBCL cells to rituximab. Br J Haematol 134:475-84, 2006

49. Tuncyurek P, Mayer JM, Klug F, Dillmann S, Henne-Bruns D, Keller F, Stracke S: Everolimus and mycophenolate mofetil sensitize human pancreatic cancer cells to gemcitabine in vitro: a novel adjunct to standard chemotherapy? Eur Surg Res 39:380-7, 2007

50. Treeck O, Wackwitz B, Haus U, Ortmann O: Effects of a combined treatment with mTOR inhibitor RAD001 and tamoxifen in vitro on growth and apoptosis of human cancer cells. Gynecol Oncol 102:292-9, 2006

51. Sieghart W, Fuereder T, Schmid K, Cejka D, Werzowa J, Wrba F, Wang X, Gruber D, Rasoul-Rockenschaub S, Peck-Radosavljevic M, Wacheck V: Mammalian target of rapamycin pathway activity in hepatocellular carcinomas of patients undergoing liver transplantation. Transplantation 83:425-32, 2007

52. Haritunians T, Mori A, O'Kelly J, Luong QT, Giles FJ, Koeffler HP: Antiproliferative activity of RAD001 (everolimus) as a single agent and combined with other agents in mantle cell lymphoma. Leukemia 21:333-9, 2007

53. Novartis: Investigator Brochure RAD001. 2009

54. Boulay A, Zumstein-Mecker S, Stephan C, Beuvink I, Zilbermann F, Haller R, Tobler S, Heusser C, O'Reilly T, Stolz B, Marti A, Thomas G, Lane HA: Antitumor efficacy of intermittent treatment schedules with the rapamycin derivative RAD001 correlates with prolonged inactivation of ribosomal protein S6 kinase 1 in peripheral blood mononuclear cells. Cancer Res 64:252-61, 2004

55. Lane DP, Hupp TR: Drug discovery and p53. Drug Discov Today 8:347-55, 2003

56. Tabernero J, Kaye S: [Value of inhibition of signal transduction and epigenetic therapy concepts in cancer therapy]. Onkologie 28 Suppl 4:43-7, 2005

57. Fouladi M, Laningham F, Wu J, O'Shaughnessy MA, Molina K, Broniscer A, Spunt SL, Luckett I, Stewart CF, Houghton PJ, Gilbertson RJ, Furman WL: Phase I study of everolimus in pediatric patients with refractory solid tumors. J Clin Oncol 25:4806-12, 2007

58. Ryan AM, Eppler DB, Hagler KE, Bruner RH, Thomford PJ, Hall RL, Shopp GM, O'Neill CA: Preclinical safety evaluation of rhuMAbVEGF, an antiangiogenic humanized monoclonal antibody. Toxicol Pathol 27:78-86, 1999

59. Ferrara N, Chen H, Davis-Smyth T, Gerber HP, Nguyen TN, Peers D, Chisholm V, Hillan KJ, Schwall RH: Vascular endothelial growth factor is essential for corpus luteum angiogenesis. Nat Med 4:336-40, 1998

60. Gordon MS, Margolin K, Talpaz M, Sledge GW, Jr., Holmgren E, Benjamin R, Stalter S, Shak S, Adelman D: Phase I safety and pharmacokinetic study of recombinant human anti-vascular endothelial growth factor in patients with advanced cancer. J Clin Oncol 19:843-50, 2001

61. Margolin K, Gordon MS, Holmgren E, Gaudreault J, Novotny W, Fyfe G, Adelman D, Stalter S, Breed J: Phase Ib trial of intravenous recombinant humanized monoclonal antibody to vascular endothelial growth factor in combination with chemotherapy in patients with advanced cancer: pharmacologic and long-term safety data. J Clin Oncol 19:851-6, 2001

62. Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, Griffing S, Bergsland E: Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol 21:60-5, 2003

63. Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, Nemunaitis JJ, Jablons DM, Langer CJ, DeVore RF, 3rd, Gaudreault J, Damico LA, Holmgren E, Kabbinavar F: Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. J Clin Oncol 22:2184-91, 2004

64. Potthast S, Schulte A, Kos S, Aschwanden M, Bilecen D: Blood oxygenation level-dependent MRI of the skeletal muscle during ischemia in patients with peripheral arterial occlusive disease. Rofo 181:1157-61, 2009

65. Willett CG, Duda DG, di Tomaso E, Boucher Y, Ancukiewicz M, Sahani DV, Lahdenranta J, Chung DC, Fischman AJ, Lauwers GY, Shellito P, Czito BG, Wong TZ, Paulson E, Poleski M, Vujaskovic Z, Bentley R, Chen HX, Clark JW, Jain RK: Efficacy, safety, and biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: a multidisciplinary phase II study. J Clin Oncol 27:3020-6, 2009

66. Ludescher B, Machann J, Eschweiler GW, Vanhofen S, Maenz C, Thamer C, Claussen CD, Schick F: Correlation of fat distribution in whole body MRI with generally used anthropometric data. Invest Radiol 44:712-9, 2009

67. Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, Shenkier T, Cella D, Davidson NE: Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 357:2666-76, 2007

68. D'Adamo DR, Anderson SE, Albritton K, Yamada J, Riedel E, Scheu K, Schwartz GK, Chen H, Maki RG: Phase II study of doxorubicin and bevacizumab for patients with metastatic soft-tissue sarcomas. J Clin Oncol 23:7135-42, 2005

69. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N: VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nat Med 5:623-8, 1999

70. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, Johnson DH: Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 355:2542-50, 2006

71. Glade Bender JL, Adamson PC, Reid JM, Xu L, Baruchel S, Shaked Y, Kerbel RS, Cooney-Qualter EM, Stempak D, Chen HX, Nelson MD, Krailo MD, Ingle AM, Blaney SM, Kandel JJ, Yamashiro DJ: Phase I trial and pharmacokinetic study of bevacizumab in pediatric patients with refractory solid tumors: a Children's Oncology Group Study. J Clin Oncol 26:399-405, 2008

72. Vignot S, Faivre S, Aguirre D, Raymond E: mTOR-targeted therapy of cancer with rapamycin derivatives. Ann Oncol 16:525-37, 2005

73. Sandsmark DK, Pelletier C, Weber JD, Gutmann DH: Mammalian target of rapamycin: master regulator of cell growth in the nervous system. Histol Histopathol 22:895-903, 2007

74. Franz DN, Leonard J, Tudor C, Chuck G, Care M, Sethuraman G, Dinopoulos A, Thomas G, Crone KR: Rapamycin causes regression of astrocytomas in tuberous sclerosis complex. Ann Neurol 59:490-8, 2006

75. Galanis E, Buckner JC, Maurer MJ, Kreisberg JI, Ballman K, Boni J, Peralba JM, Jenkins RB, Dakhil SR, Morton RF, Jaeckle KA, Scheithauer BW, Dancey J, Hidalgo M, Walsh DJ: Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. J Clin Oncol 23:5294-304, 2005

76. Atkins MB, Hidalgo M, Stadler WM, Logan TF, Dutcher JP, Hudes GR, Park Y, Liou SH, Marshall B, Boni JP, Dukart G, Sherman ML: Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. J Clin Oncol 22:909-18, 2004

77. Chan S, Scheulen ME, Johnston S, Mross K, Cardoso F, Dittrich C, Eiermann W, Hess D, Morant R, Semiglazov V, Borner M, Salzberg M, Ostapenko V, Illiger HJ, Behringer D, Bardy-Bouxin N, Boni J, Kong S, Cincotta M, Moore L: Phase II study of temsirolimus (CCI-779), a novel inhibitor of mTOR, in heavily pretreated patients with locally advanced or metastatic breast cancer. J Clin Oncol 23:5314-22, 2005

78. Schuetze S, Baker L, Maki R: Sirolimus reduced tumor-related morbidity and resulted in biochemical and radiographic response in patiens with progressive sarcoma, in Grunberg S (ed): ASCO. Atlanta, GA, 2006, pp 520s

79. Rao RD, Buckner JC, Sarkaria JN: Mammalian target of rapamycin (mTOR) inhibitors as anti-cancer agents. Curr Cancer Drug Targets 4:621-35, 2004

80. Dasgupta B, Yi Y, Chen DY, Weber JD, Gutmann DH: Proteomic analysis reveals hyperactivation of the mammalian target of rapamycin pathway in

neurofibromatosis 1-associated human and mouse brain tumors. Cancer Res 65:2755-60, 2005

81. Johannessen CM, Reczek EE, James MF, Brems H, Legius E, Cichowski K: The NF1 tumor suppressor critically regulates TSC2 and mTOR. Proc Natl Acad Sci U S A 102:8573-8, 2005

82. Johansson G, Mahller YY, Collins MH, Kim MO, Nobukuni T, Perentesis J, Cripe TP, Lane HA, Kozma SC, Thomas G, Ratner N: Effective in vivo targeting of the mammalian target of rapamycin pathway in malignant peripheral nerve sheath tumors. Mol Cancer Ther 7:1237-45, 2008

83. Hegedus B, Banerjee D, Yeh TH, Rothermich S, Perry A, Rubin JB, Garbow JR, Gutmann DH: Preclinical cancer therapy in a mouse model of neurofibromatosis-1 optic glioma. Cancer Res 68:1520-8, 2008

84. Johannessen CM, Johnson BW, Williams SM, Chan AW, Reczek EE, Lynch RC, Rioth MJ, McClatchey A, Ryeom S, Cichowski K: TORC1 is essential for NF1-associated malignancies. Curr Biol 18:56-62, 2008

85. O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, Lane H, Hofmann F, Hicklin DJ, Ludwig DL, Baselga J, Rosen N: mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 66:1500-8, 2006

86. Kranenburg O, Gebbink MF, Voest EE: Stimulation of angiogenesis by Ras proteins. Biochim Biophys Acta 1654:23-37, 2004

87. Rak J, Mitsuhashi Y, Bayko L, Filmus J, Shirasawa S, Sasazuki T, Kerbel RS: Mutant ras oncogenes upregulate VEGF/VPF expression: implications for induction and inhibition of tumor angiogenesis. Cancer Res 55:4575-80, 1995

88. Kawachi Y, Xu X, Ichikawa E, Imakado S, Otsuka F: Expression of angiogenic factors in neurofibromas. Exp Dermatol 12:412-7, 2003

89. Angelov L, Salhia B, Roncari L, McMahon G, Guha A: Inhibition of angiogenesis by blocking activation of the vascular endothelial growth factor receptor 2 leads to decreased growth of neurogenic sarcomas. Cancer Res 59:5536-41, 1999

90. Messiaen LM, Callens T, Mortier G, Beysen D, Vandenbroucke I, Van Roy N, Speleman F, Paepe AD: Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. Hum Mutat 15:541-55, 2000

91. De Raedt T, Brems H, Wolkenstein P, Vidaud D, Pilotti S, Perrone F, Mautner V, Frahm S, Sciot R, Legius E: Elevated risk for MPNST in NF1 microdeletion patients. Am J Hum Genet 72:1288-92, 2003

92. Perry A, Roth KA, Banerjee R, Fuller CE, Gutmann DH: NF1 deletions in S-100 protein-positive and negative cells of sporadic and neurofibromatosis 1 (NF1)-associated plexiform neurofibromas and malignant peripheral nerve sheath tumors. Am J Pathol 159:57-61, 2001

93. Dombi E, Solomon J, Gillespie AJ, Fox E, Balis FM, Patronas N, Korf BR, Babovic-Vuksanovic D, Packer RJ, Belasco J, Goldman S, Jakacki R, Kieran M, Steinberg SM, Widemann BC: NF1 plexiform neurofibroma growth rate by volumetric MRI: relationship to age and body weight. Neurology 68:643-7, 2007

94. Solomon J, Warren K, Dombi E, Patronas N, Widemann B: Automated detection and volume measurement of plexiform neurofibromas in neurofibromatosis 1 using magnetic resonance imaging. Comput Med Imaging Graph 28:257-65, 2004

95. Loomba R, Rowley A, Wesley R, Liang TJ, Hoofnagle JH, Pucino F, Csako G: Systematic review: the effect of preventive lamivudine on hepatitis B reactivation during chemotherapy. Ann Intern Med 148:519-28, 2008

96. Yeo W, Lam KC, Zee B, Chan PS, Mo FK, Ho WM, Wong WL, Leung TW, Chan AT, Ma B, Mok TS, Johnson PJ: Hepatitis B reactivation in patients with hepatocellular carcinoma undergoing systemic chemotherapy. Ann Oncol 15:1661-6, 2004

97. Albritton K, Rankin C, Coffin M, Ratner N, Budd G, Schuetze S, Randall L, DeClue J, Borden E: Phase II study of erlotinib in metastatic or unresectable malignant peripheral nerve sheath tumors (MPNSTs), ASCO. Atlanta, GA, 2006, pp 524s

98. Simon R: Optimal two-stage designs for phase II clinical trials. Control Clin Trials 10:1-10, 1989

99. Therasse P, Arbuck S, Eisenhauer E, Wanders J, Kaplan R, Rubinstein L, Verweij J, Van Glabbeke M, Van Oosterom T, Chrisitan M, Gwyther S: New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92:205-216, 2000

100. Miller AB, Hoogstraten B, Staquet M, Winkler A: Reporting results of cancer treatment. Cancer 47:207-14, 1981

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: P450 DRUG INTERACTION TABLE

(as per http://medicine.iupui.edu/clinpharm/ddis/table.aspx; from Section 6.4, Interactions, Investigator's Brochure, Afinitor®/Votubia® (everolimus) (07-Nov-2011)

Appendix II: P450 Drug Interaction Table (as per http://medicine.iupui.edu/clinpharm/ddis/table.aspx; from Section 6.4, Interactions, Investigator's Brochure, Afinitor®/Votubia® (RAD001) (07-Nov-2011)

SUBSTRATES

1A2	2B6	2C8	2C9	2C19	2D6	2E1	3A4,5,7
amitriptyline caffeine[2] clomipramine clozapine cyclobenzaprine estradiol fluvoxamine haloperidol imipramine N-DeMe mexiletine naproxen olanzapine ondansetron phenacetin[1]→ acetaminophen→NAPQI propranolol riluzole ropivacaine tacrine[2] theophylline[2] tizanidine verapamil (R)warfarin zileuton zolmitriptan	bupropion[1] cyclophosphamide efavirenz[1] ifosphamide methadone sorafenib	amodiaquine[2] cerivastatin paclitaxel repaglinide sorafenib torsemide	**NSAIDs:** diclofenac[1] ibuprofen lornoxicam meloxicam S-naproxen→Nor piroxicam suprofen **Oral Hypoglycemic Agents:** tolbutamide[1] glipizide **Angiotensin II Blockers:** losartan irbesartan **Sulfonylureas:** glyburide glibenclamide glipizide glimepiride tolbutamide amitriptyline celecoxib fluoxetine fluvastatin glyburide nateglinide phenytoin-4-OH2 rosiglitazone tamoxifen torsemide S-warfarin[1]	**PPIs:** lansoprazole omeprazole2 pantoprazole rabeprazole **Anti-epileptics:** diazepam→Nor phenytoin(O) S-mephenytoin[1] phenobarbitone amitriptyline carisoprodol citalopram chloramphenicol clomipramine clopidogrel cyclophosphamide hexobarbital imipramine N-DeME indomethacin R-mephobarbital moclobemide nelfinavir nilutamide primidone progesterone proguanil propranolol teniposide R-warfarin→8-OH	tamoxifen: **TAMOXIFEN GUIDE** **Beta Blockers:** carvedilol S-metoprolol propafenone timolol **Antidepressants:** amitriptyline clomipramine desipramine fluoxetine imipramine paroxetine venlafaxine **Antipsychotics:** haloperidol perphenazine risperidone→9-OH thioridazine zuclopenthixol alprenolol amphetamine aripiprazole atomoxetine bufuralol[1] chlorpheniramine chlorpromazine clonidine codeine (→O-desMe) debrisoquine[2] dexfenfluramine dextromethorphan[1] donepezil duloxetine encainide flecainide fluvoxamine lidocaine metoclopramide	**Anesthetics:** enflurane halothane isoflurane methoxyflurane sevoflurane acetaminophen→NAPQI aniline2 benzene chlorzoxazone1 ethanol N,N-dimethylformamide theophylline→8-OH	**Macrolide antibiotics:** clarithromycin erythromycin[2] (not 3A5) NOT azithromycin telithromycin **Anti-arrhythmics:** quinidine→3-OH (not 3A5) **Benzodiazepines:** alprazolam diazepam→3OH midazolam[1] triazolam[2] **Immune Modulators:** cyclosporine tacrolimus (FK506) **HIV Antivirals:** indinavir nelfinavir ritonavir saquinavir **Prokinetic:** cisapride **Antihistamines:** astemizole chlorpheniramine terfenadine[2] **Calcium Channel Blockers:** amlodipine diltiazem felodipine lercanidipine nifedipine[2] nisoldipine nitrendipine verapamil

methoxyamphetamine
mexiletine
minaprine
nebivolol
nortriptyline
ondansetron
oxycodone
perhexiline
phenacetin
phenformin
promethazine
propranolol
sparteine
tramadol

HMG CoA Reductase Inhibitors:
atorvastatin
cerivastatin
lovastatin
NOT pravastatin
NOT rosuvastatin
simvastatin

Steroid 6beta-OH:
estradiol
hydrocortisone
progesterone
testosterone[1]

Miscellaneous:
alfentanil
aprepitant
aripiprazole
boceprevir
buspirone
cafergot
caffeine→TMU
cilostazol
cocaine
codeine-N-demethylation
dapsone
dexamethasone
dextromethorphan[2]
docetaxel
domperidone
eplerenone
fentanyl
finasteride
gleevec
haloperidol
irinotecan
LAAM
lidocaine
methadone
nateglinide
ondansetron
pimozide
propranolol
quetiapine
quinine
risperidone
salmeterol
sildenafil
sirolimus
sorafenib

sunitinib
tamoxifen
taxol
telaprevir
terfenadine
torisel
trazodone
vincristine
zaleplon
ziprasidone
zolpidem

INHIBITORS

Inhibitors compete with other drugs for a particular enzyme thus affecting the optimal level of metabolism of the substrate drug which in many cases affect the individual's response to that particular medication, e.g. making it ineffective.

■A **Strong inhibitor** is one that causes a > 5-fold increase in the plasma AUC values or more than 80% decrease in clearance.
■A **Moderate inhibitor** is one that causes a > 2-fold increase in the plasma AUC values or 50-80% decrease in clearance.
■A **Weak inhibitor** is one that causes a > 1.25-fold but < 2-fold increase in the plasma AUC values or 20-50% decrease in clearance.
■All other inhibitors.

FDA preferred[1] and acceptable[2] **inhibitors** for in vitro experiments.*

1A2	2B6	2C8	2C9	2C19	2D6	2E1	3A4,5,7
■fluvoxamine ■ciprofloxacin ■cimetidine amiodarone fluoroquinolones furafylline[1] interferon methoxsalen mibefradil ticlopidine	thiotepa ticlopidine[2]	■gemfibrozil[2] ■trimethoprim[2] glitazones montelukast[1] quercetin[1]	■fluconazole[2] ■amiodarone fenofibrate fluvastatin fluvoxamine[2] isoniazid lovastatin phenylbutazone probenicid sertraline sulfamethoxazole sulfaphenazole[1] teniposide voriconazole zafirlukast	PPIs: lansoprazole omeprazole2 pantoprazole rabeprazole chloramphenicol cimetidine felbamate fluoxetine fluvoxamine indomethacin ketoconazole modafinil oxcarbazepine probenicid ticlopidine2 topiramate	■bupropion ■cinacalcet ■fluoxetine ■paroxetine ■quinidine[1] ■duloxetine ■sertraline ■terbinafine ■amiodarone ■cimetidine celecoxib chlorpheniramine chlorpromazine citalopram clemastine clomipramine cocaine diphenhydramine doxepin doxorubicin escitalopram halofantrine	diethyl-dithiocarbamate[2] disulfiram	HIV Antivirals: ■indinavir ■nelfinavir ■ritonavir ■clarithromycin ■itraconazole[1] ■ketoconazole[1] ■nefazodone ■saquinavir ■telithromycin ■aprepitant ■erythromycin ■fluconazole ■grapefruit juice ■verapamil[2] ■diltiazem **voriconazole** ■cimetidine amiodarone NOT azithromycin chloramphenicol boceprevir

APPENDIX III: CLINICALLY RELEVANT DRUG INTERACTIONS: SUBSTRATES, INDUCERS INHIBITORS OF PGP AND PGP/CYP3A DUAL INHIBITORS

Table 6-12 Clinically relevant drug interactions: substrates, inducers, inhibitors of PgP and PgP/CYP3A dual inhibitors

Substrates
Digoxin, fexofenadine, indinavir, vincristine, colchicine, topotecan, paclitaxel, talinolol
Inducers
Rifampin, St John's wort
PgP Inhibitors and PgP/CYP3A Dual Inhibitors
amiodarone, captopril, carvedilol, clarithromycin, conivaptan, diltiazem, dronedarone, elacridar, erythromycin, felodipine, fexofenadine, ginkgo (ginkgo biloba), indinavir, itraconazole, , lopinavir, mibefradil, milk thistle (silybum marianum), nifedipine, nitrendipine, quercetin, quinidine, ranolazine, ritonavir, saquinavir, Schisandra chinensis, St John's wort (hypericum perforatum), talinolol, telmisartan, tipranavir, valspodar, verapamil
Reference: Internal Clinical Pharmacology Drug-drug interaction (DDI) memo, updated Oct 2, 2011, which summarizes DDI data from three sources including the FDA's "Guidance for Industry, Drug Interaction Studies", the University of Washington's Drug Interaction Database, and Indiana University School of Medicine's Drug Interaction Table. *Additional Reference: Baltes et al. Fundamental & Clinical Pharmacology 19 (2005) 511–529

APPENDIX IV: 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:	

SARC016 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.

Everolimus

- Your dose of everolimus is _____
- You will take everolimus once per day, every day of each 28 day cycle.
- Take each dose at the same time each day, preferably in the morning. You should take the everolimus either in a fasting state (no food one hour before or two hours after meals) or with a light fat-free meal. If you choose one method over the other you should use that method every day you take everolimus.
- If you miss a dose, you may still take it up to 6 hours after the time you would normally take it. If more than 6 hours have elapsed, you should skip the dose for that day. The next day, you should take the everolimus at the usual time. Do not take 2 doses to make up for the missed dose.
- If you vomit your dose of everolimus, 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 everolimus. You should avoid grapefruit, Seville oranges, or star fruit and the juices of these fruits, and St. John's Wort while you are taking everolimus, as these items may change how your body handles everolimus.
- It is important that you maintain good oral hygiene (mouth care) while you are taking everolimus to help prevent inflammation of the mouth tissues.
- You should not receive live vaccines or have close contact with those who have received them while you are taking everolimus.
- Bring any unused everolimus, and this diary to each clinic visit. The study staff will make sure you have an adequate supply of everolimus 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

DOSING LOG

	Everolimus: For each dose, take _____. Please indicate the date, time, amount taken and any comments.		
	Date & Time	**Amount Taken**	**Comments**
Ex:	6/1/09 4:30 pm	1 pill	Vomited dose
Day 1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			

DOSING LOG Continued…

	Date & Time	Amount Taken	Comments
Ex:	6/1/09	1 pill	Vomited dose
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			

APPENDIX V: HEPATITIS SCREENING/MONITORING/TREATMENT

Reactivation of Hepatitis B (HBV) has been observed in patients with cancer receiving chemotherapy. Sporadic cases of Hepatitis B reactivation have also been seen in this setting with everolimus. Use of antivirals during anti-cancer therapy has been shown to reduce the risk of Hepatitis B virus reactivation and associated morbidity and mortality. A detailed assessment of Hepatitis B/C medical history and risk factors must be done for all patients at screening, with testing performed prior to the first dose of everolimus.

Testing for hepatitis B viral load and serologic markers: HBV-DNA, HBsAg, HBs Ab, and HBc Ab and HCV RNA PCR are required at screening for all patients in the following risk categories:

- All patients who currently live in (or have lived in) Asia, Africa, Central and South America, Eastern Europe, Spain, Portugal, and Greece.
- Patients with any of the following risk factors:
  - known or suspected past hepatitis B infection,
  - blood transfusion(s) prior to 1990,
  - current or prior IV drug users,
  - current or prior dialysis,
  - household contact with hepatitis B infected patient(s),
  - current or prior high-risk sexual activity,
  - body piercing or tattoos,
  - mother known to have hepatitis B
  - history suggestive of hepatitis B infection, e.g., dark urine, jaundice, right upper quadrant pain.

Management of Hepatitis reactivation

In cancer patients with hepatitis B, whether carriers or in chronic state, use of antivirals during anticancer therapy has been shown to reduce the risk of hepatitis B virus (HBV) reactivation and associated HBV morbidity and mortality.

Monitoring and prophylactic treatment for hepatitis B reactivation

The tables below provide details of monitoring and prophylactic therapy according to the baseline results of viral load and serologic markers testing.

Action to be taken for positive baseline hepatitis B results

Test	Result	Result	Result	Result	Result
HBV-DNA	+	+ or -	-	-	-
HBsAg	+ or -	+	-	-	-
HBs Ab	+ or -	+ or -	+	+ or -	-

Test	Result	Result	Result	Result	Result
			and no prior HBV vaccination		or + with prior HBV vaccination
HBc Ab	+ or -	+ or -	+ or -	+	-
Recommendation	Prophylaxis treatment should be started 1-2 weeks prior to first dose of study drug Monitor HBV-DNA approximately every 4 weeks		No prophylaxis Monitor HBV-DNA approximately every 4 weeks		No specific action

Antiviral prophylaxis therapy should continue for at least 4 weeks after last dose of study drug.

For patients who have already received study drug prior to the approval of the amendment, the same process should be followed at the patient's next visit. The first HBV-DNA result would be regarded as baseline.

For hepatitis B reactivation, definition and management guidelines, see Table 3-3 Guidelines for management of hepatitis B.

Guidelines for management of hepatitis B

HBV reactivation (with or without clinical signs and symptoms)*

For patients with baseline results: Positive HBV-DNA OR positive HBsAg ---------- reactivation is defined as: [Increase of 1 log in HBV-DNA relative to baseline HBV-DNA value OR new appearance of measurable HBV-DNA] AND ALT elevation x 5 ULN	Treat: Start a second antiviral AND Interrupt study drug administration until resolution: ≤ grade 1 ALT (or baseline ALT, if > grade 1) and ≤ baseline HBV-DNA levels If resolution occurs within ≤ 28 days study drug should be re-started at one dose lower, if available. If the patient is already receiving the lowest dose of study drug according to the protocol, the patient should restart at the same dose after resolution. Both antiviral therapies should continue at least 4 weeks after last dose of study drug. If resolution occurs > 28 days Patients should discontinue study drug but continue both antiviral therapies at least 4 weeks after last dose of study drug.
For patients with baseline results: Negative HBV-DNA and HBsAg AND [Positive HBs Ab (with no prior history of vaccination against HBV), OR positive	Treat : Start first antiviral medication AND Interrupt study drug administration until resolution: ≤ baseline HBV-DNA levels If resolution occurs within ≤ 28 days study drug should be re-started at one dose lower, if available. If the patient is already receiving the lowest dose of study drug according to the protocol, the patient should restart at the same

HBc Ab] --- reactivation is defined as: New appearance of measurable HBV-DNA	dose after resolution. Antiviral therapy should continue at least 4 weeks after last dose of study drug. If resolution occurs > 28 days Patients should discontinue study drug but continue antiviral therapy at least 4 weeks after last dose of study drug.

* All reactivations of hepatitis B are to be recorded as grade 3 (CTCAE v 3.0 Metabolic Laboratory/Other: Viral Re-activation), unless considered life threatening by the investigator; in which case they should be recorded as grade 4 (CTCAE v 3.0 Metabolic Laboratory/Other: Viral Re-activation). Date of viral reactivation is the date on which **both** DNA and ALT criteria were met (e.g. for a patient who was HBV-DNA positive on 01-JAN-10 and whose ALT reached ≥ 5 × ULN on 01-APR-10, the date of viral reactivation is 01-APR-10).

Monitoring for hepatitis C

The following two categories of patients should be monitored every 4 weeks for HCV reactivation:

- Patients with detectable HCV RNA-PCR test at baseline.
- Patients known to have a history of HCV infection, despite a negative viral load test at baseline (including those that were treated and are considered 'cured')

For definition of hepatitis C reactivation and the management guidelines, see the table below with guidelines for management of hepatitis C.

Guidelines for management of hepatitis C

HCV reactivation*	
For patients with baseline results: Detectable HCV-RNA, reactivation is defined as: ALT elevation x 5 ULN	Discontinue study drug
For patients with baseline results: Knowledge of past hepatitis C infection with no detectable HCV-RNA, reactivation is defined as: New appearance of detectable HCV-RNA	Discontinue study drug

* All reactivations of hepatitis C are to be recorded as grade 3 (CTCAE v3.0 Metabolic Laboratory/Other: Viral Re-activation), unless considered life threatening by the investigator; in which case they should be recorded as grade 4 (CTCAE v 3.0 Metabolic Laboratory/Other: Viral Re-activation).

APPENDIX VI: 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