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A Phase 2 Study of Dasatinib in Combination with Everolimus for Children with Gliomas Harboring *PDGFR*/Alterations

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1.0 Protocol Summary

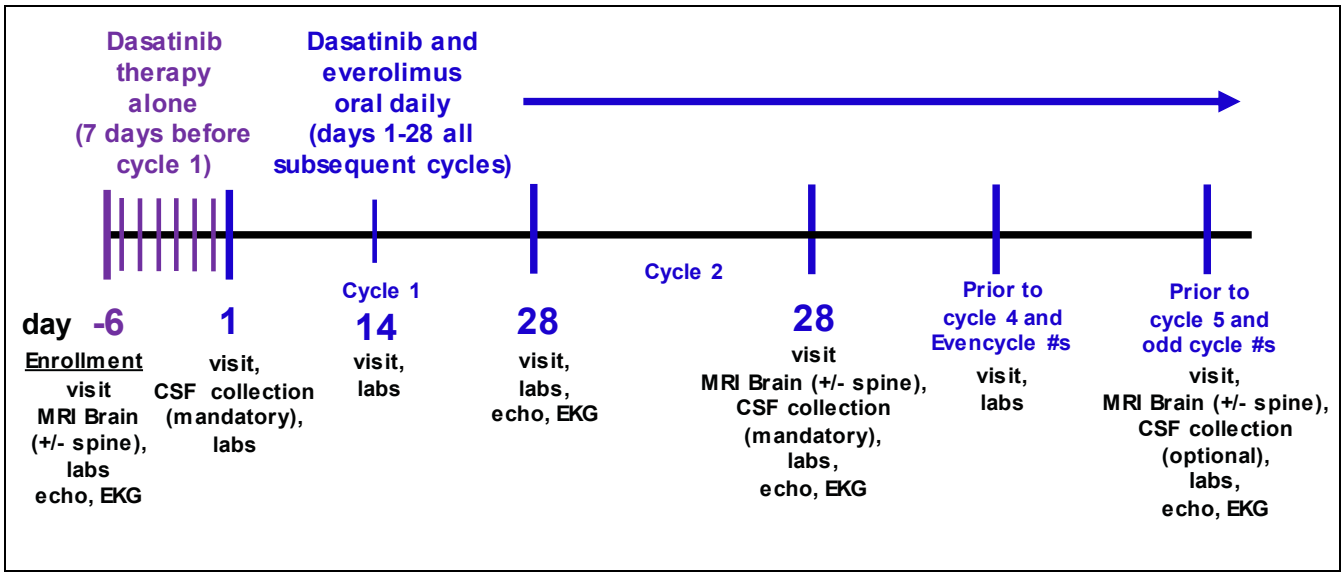
Outcomes for children with brain tumors have lagged and brain tumors have become the leading cause of cancer-related mortality in younger patients.[1] Because the blood brain barrier (BBB) restricts access to the central nervous system (CNS) to all but 5% of chemical compounds screened for drug development, utilization of targeted therapies in CNS tumors remains challenging.[2] Furthermore, traditional early-phase studies in pediatric neuro-oncology have not been designed to select patients based on individual tumor biology. Incorporating a precision medicine approach that both takes into account individual tumor biology and optimizes BBB drug penetration is a promising approach to improve outcomes for this devastating group of tumors.

This phase 2 trial will evaluate the activity of dasatinib in combination with everolimus for children with gliomas harboring *PDGFR* or alterations, including newly diagnosed high-grade glioma or DIPG after radiation (stratum A) and recurrent/progressive glioma (grade II-IV, including DIPG) (stratum B). This approach involves incorporating a precision medicine approach (dasatinib targeting PDGFR/) with BBB optimization (everolimus inhibition of Bcrp1 and P-gp and further RTK/PI3K pathway targeting). Eligibility will be determined by documentation of PDGFR or pathway alterations through tumor DNA and RNA sequencing. We will provide CLIA- certified molecular profiling of tumors through the University of Michigan PEDS-MIONCOSEQ study (HUM00056496). We will also accept CLIA-certified profiling (DNA/RNA sequencing) from private sequencing facilities.

The primary objective of this study is to test and estimate the efficacy of the proposed regimen. The planned starting dose for dasatinib is the dose currently undergoing phase 2 trial in children with refractory solid tumors (AALL1131, NCT02883049), 60 mg/m^2 orally twice daily. No dose escalation is planned. The planned starting dose for everolimus is 2/3 the current FDA-approved starting dose for children with sub-ependymal giant cell astrocytoma (SEGA) (3.0 mg/m^2), with titration of dosing after first cycle to keep everolimus trough level of 5-15 ug/ml. Both agents will be taken daily for 28 day cycles. Cycles will be repeated every 28 days and patients may receive up to 24 cycles. Disease evaluation by MRI will be performed every two cycles. Drug levels of both agents will be monitored after first month; and serum level of everolimus will be monitored monthly throughout therapy to adjust dosing to trough concentration to 5-15 ng/ml. High b-value diffusion weighted imaging (DWI) will be performed at the time of clinical MRIs to evaluate its utility as an imaging biomarker for therapy response.

Cerebrospinal fluid (CSF) and plasma dasatinib pharmacokinetics will be performed by the University of Michigan to determine whether blood-CSF permeability of dasatinib is impacted by

dual therapy with everolimus. Prior to first cycle, dasatinib will be taken as mono-therapy for 1 week followed by CSF collection in order to establish mono-therapy dasatinib CSF level. After cycle 2, CSF will be collected to establish dual-therapy dasatinib level (while on everolimus). Additionally, CSF collected at these time points will be used to explore CSF cell-free tumor DNA



2.0 Objectives and Scientific Aims

2.1 Primary Objective

2.1.1 STRATUM A: To determine the 1 year and 2 year progression-free and overall survival rates in patients receiving dasatinib in combination with everolimus as an adjuvant therapy following surgery and radiation therapy for children with newly diagnosed high-grade glioma or DIPG harboring *PDGFR* or alterations.

2.1.2 STRATUM B: To determine the overall response rate after 2 cycles of dasatinib in combination with everolimus in children with refractory or recurrent glioma (grade II-IV, including DIPG) with *PDGFR* or alterations.

2.2 Exploratory Objectives

2.2.1 To define and describe the toxicities of dasatinib and everolimus administered as dual therapy.

2.2.2 To prospectively evaluate high b-value diffusion weighted imaging (DWI) as an imaging biomarker for dasatinib and everolimus activity.

2.2.3 To prospectively evaluate CSF and plasma cell-free tumor DNA (ct-DNA) as a marker of disease activity in refractory glioma with *PDGFR*/alterations.

2.2.4 To prospectively evaluate CSF dasatinib level as (1) a marker of blood-CSF permeability of dasatinib and (2) a predictor of treatment response.

3.0 Background and Rationale

Although outcomes for children and young adults with cancer have steadily improved over the past half-century, outcomes for children with brain tumors have lagged and brain tumors have become the leading cause of cancer-related mortality in younger patients.[1] This is in large part due to the difficulty of employing many of the effective oncology treatments in this patient population.[1,3,4] Because the blood brain barrier (BBB) restricts access to the central nervous system (CNS) to all but 5% of chemical compounds screened for drug development, utilization of targeted therapies in CNS tumors remains challenging.[2] Furthermore, traditional early-phase studies in pediatric neuro-oncology have not been designed to select patients based on individual tumor biology. New therapies for malignant glioma are urgently needed. Incorporating a precision medicine approach that both takes into account individual tumor biology and optimizes BBB drug penetration is a promising approach to improve outcomes for this devastating group of tumors. This trial will evaluate the activity of dasatinib in combination with everolimus for children with malignant gliomas harboring *PDGFR* or alterations, including newly diagnosed high-grade (grade III-IV) glioma after radiation (stratum A) and recurrent/progressive grade II-IV glioma (stratum B).

3.1 Precision Medicine for Pediatric Brain Tumors

By histology, adult and pediatric brain tumors are identical; but at the molecular level, they are dissimilar tumors.[3,5] Recurrent mutations and gene expression profiles of pediatric brain tumors are clearly distinct from their adult counterparts.[3,6,7] In fact, molecular characterization of pediatric brain tumors has documented key differences *among* subgroups of the same tumor, as separated by age and location.[3,5,8] Treatment responses may differ as well, with younger children responding to chemotherapy better than older children for certain brain tumors.[9] Effective therapies for children and young adults with higher risk or refractory brain tumors are in urgent need, ideally based on the unique biology of each tumor.

In contrast to analyses of adult tumors, recent surveys of pediatric tumors have shown that most develop from relatively few mutational events, many of which may be targetable with personalized clinically available agents.[3,10] With the increasing use of tumor molecular profiling at many tertiary pediatric hospitals, multiple studies have demonstrated the feasibility of incorporating personalized genomic characterization into the management of higher-risk, refractory, or rare pediatric tumors.[11-13] Precision medicine is therefore an exciting potential therapeutic area for younger patients with brain tumors.

3.2 *PDGFR* and Alterations in Pediatric Gliomas

Recent work has documented the mutation, amplification and up-regulation of *PDGFRA* in a significant subset (15-39%) of pediatric patients with high-grade glioma (HGG).[7,14] In collaboration with Chris Jones (Institute for Cancer Research, London, UK), we recently analyzed an integrated genomic dataset from pediatric HGG patients (n=290) using multiple pediatric datasets and sequencing platforms. We found activating *PDGFR* alterations in 41 (14.1%) patients. In multivariate analysis, *PDGFRA* mutation was correlated with worse prognosis (P =

0.026), and by Kaplan-Meier analysis, non-brainstem HGG with *PDGFRA* amplification carried a worse prognosis than non-brainstem HGG without *PDGFRA* amplification (P = 0.021), thus demonstrating its potential role as a significant tumor driver in pediatric HGG.[15]

Additionally, our group recently performed an observational consecutive case-series involving children and young adults with brain tumors designated by their treating neuro-oncologist to be high risk (>25% chance for treatment failure) (mean age 9.7 years, range age 0-39, with only 3 patients over 21). Participants underwent integrative clinical exome (tumor and germline DNA) and transcriptome (tumor RNA) sequencing using clinically integrated sequencing that was performed according to previous published CLIA-certified methodology.[11,17] Of the 50 enrolled patients, eighteen patients (36%) had relapsed or refractory tumors (of which we sequenced the original tumor in 12 cases), and 13 patients (26%) had metastatic tumors. Sequencing revealed potentially actionable genomic alterations (as defined by our group in Mody et al, JAMA, 2015) Sequencing revealed a potentially actionable germline or tumor alterations in 25 (63%) tumors, of which 21 (53%) resulted in an impact on treatment or change of diagnosis. Seventeen of 20 patients (85%) with glial tumors were found to have a potentially actionable result, which resulted in change of therapy in 14 (70%) patients. Six (15%) patients who underwent therapy changes had potential clinical benefit (partial response or stable disease greater than 6 months on therapy). Interestingly, 9 of 20 (45%) of glial tumors harbored genomic alteration (activating mutation or amplification) in the growth factor receptors PDGFR or FGFR or their ligands (PDGF, FGF). All six alterations were associated with corresponding outlier increased expression on RNA sequencing (Koschmann et al*, Under Review*).

3.3 Targeting *PDGFR* and with TKIs and Overcoming Efflux Proteins

There are multiple orally bioavailable tyrosine kinase inhibitors (TKIs) with published safety data in pediatric cancers. While many of these were originally developed for targeting receptor tyrosine kinases in human cancers, their kinase inhibition is not specific, and many display nanomolar efficacy against PDGFRA or .[18] Some TKIs display activity active against HGG *in vitro* and *in vivo* in orthotopic animal models, and target many of the growth factor receptor pathways active in human glioma, including PDGFRA, PDGFRB, FGFR1/3, c-Kit, and VEGFR.[15,19-21] Many TKIs display favorable characteristics for BBB penetration (small size, high lipophilicity), but are substrates for the active efflux proteins P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), which significantly limits their brain penetration.[20] Importantly, recent work has shown that TKI delivery to brain tumor parenchyma may be further improved by strategies to inhibit P-gp and Bcrp1.[20,22,23] Recent pre-clinical strategies have included co-administration of TKIs with agents that inhibit these proteins, including mammalian target of rapamycin (mTOR) inhibitors.[24]

Given that efflux mechanisms appear to be the main limitation to CNS penetration of dasatinib, the concomitant use of a P-gp inhibiting pharmacologic agent (everolimus) may be beneficial. We recently reviewed this topic and described our early clinical experience with this model (Marini et al).[25] A common theme in the evaluation of targeted agents in neuro-oncology is the impediment of BBB penetration due to the presence of efflux pumps. These energy-dependent transporters are expressed on the apical surface of the cells that line the vasculature and actively efflux compounds that are able to cross the membrane via passive diffusion or other methods, thus limiting CNS accumulation of promising targeted therapies.[26] The most commonly found ABC efflux

transporters, P-gp (also known as ABCB1 or MDR1) and BRCP (also known as ABCG2), are responsible for the efflux of a wide variety of traditional chemotherapy agents (e.g., anthracyclinces, taxanes, methotrexate).[27] While small molecule inhibitors that have been developed in the last 15 years allow for targeted therapy based on molecular abnormalities, the vast majority of these molecules are also substrates for P-gp and BCRP efflux pumps. Animal models with a variety of targeted therapies have demonstrated that inhibition of the efflux pumps can result in increased concentrations of the targeted therapy into the brain.[20,24,28-31]

The concept of efflux pump inhibition to enhance CNS concentrations of the desired targeted agents has been shown to be effective pre-clinically, as well as in several patient cases.[20,24,29-32] Elacridor, a dual P-gp and BCRP inhibitor, was developed as such a pharmacokinetic modulator; however, this agent is not FDA approved or currently available clinically. There are few known, FDA-approved agents that inhibit both P-gp and BCRP, yet have minimal adverse pharmacologic effects. Everolimus, an orally bioavailable mTOR inhibitor, inhibits both P-gp and BCRP. In a preclinical study, everolimus was added to vandetanib, an oral multikinase inhibitor and P-gp and BCRP substrate with minimal CNS penetration but demonstrated *in vitro* activity in preclinical non-small cell lung cancer and brain tumor cells.[24] Concomitant administration of everolimus resulted in a 3–4-fold increase in the murine brain to plasma concentration ratio of vandetanib. Based on these findings, as well as identified targetable RET mutation, a group of investigators reported treating a patient with non-small cell lung cancer with brain metastases using concomitant everolimus and vandetanib on a study protocol (ClinicalTrials.gov #NCT01582191), ultimately achieving both a systemic and intracranial response by PET/CT and MRI, respectively.[32] Similarly, in pre-clinical models, by modulating P-gp and BCRP with the epidermal growth factor (EGFR) inhibitors erlotinib and canertinib, brain accumulation of pazopanib, a multikinase inhibitor approved for renal cell carcinoma and soft-tissue sarcoma, was increased 2–2.5-fold.[20]

Importantly, recent work has shown that dasatinib delivery to brain tumor parenchyma is primarily restricted by P-gp, and inihibition of P-gp can improve penetration. We therefore propose the use of dasatinib with the mTOR (and P-gp) inhibitor everolimus for the treatment of PDGF- and -altered pediatric brain tumors.[33]

3.4 Dasatinib

Dasatinib (SPRYCEL™, Bristol Myers Squib) is an orally bioavailable, ATP competitive small-molecular multi-targeted tyrosine kinase inhibitor.[34] Dasatinib is currently FDA approved for the treatment of patients with Philadelphia chromosome positive acute lymphoblastic leukemia. Dasatinib is active against the growth factor receptors BCR/ABL, Src, C-kit, and *PDGF*R-A/B. Dasatinib is a promising agent for pediatric HGG with PDGF pathway alterations. Dasatinib is an orally bioavailable tyrosine kinase inhibitor with ~60 fold greater inhibition of PDGFR signaling than earlier generation TKIs, such as imatinib.[36] Our group previously performed paired molecular profiling (germline / tumor / primary cell culture) and targeting of infant thalamic HGG cells (UMPED05) with amplification and outlier increased expression of PDGFRA.[15] Dasatinib inhibited proliferation most effectively. While it was originally developed for targeting the BCR-ABL gene fusion, it exhibits nanomolar range activity against PDGFRA in leukemia.[18] In the previous pediatric Phase I trial using dasatinib in patients with leukemia and solid tumors, it was found to be well tolerated.[37] Dasatinib displays moderately favorable characteristics for blood-brain barrier penetration and has demonstrated efficacy in adult patients with CNS metastases of CML,[38] where CSF concentrations (3 nMol/L and 20 nMol/L) were near the IC_{50} we obtained for

UMPED05 (50 nMol/L) and previously published tumor cell cultures (4 nMol/L).[36]

3.5 Everolimus

Everolimus (AFFINITOR™, ZORTRESS™, Novartis) is an mTOR pathway inhibitor which is FDA approved for the treatment of advanced renal cell carcinoma, pancreatic neuroendocrine tumor, and subependymal giant cell astrocytoma (SEGA), including pediatric SEGA.[39,40] Everolimus has undergone phase 1 testing in pediatric solid tumor patients and the recommended phase 2 dose was 5 mg/m^2 daily.[41] Many children with tuberous sclerosis, who harbor a germline deficit related to the mTOR pathway, develop SEGA. In a phase 3 trial in children with SEGA, efficacy of everolimus was demonstrated in 75% of patients and no patients experienced disease progression during treatment.[42] The efficacy of everolimus is currently being studied in a variety of tumors including pediatric brain tumors. Rare dose-limiting toxicities have included mucositis, infection and non-infectious pneumonitis. The starting dose for everolimus in the phase 3 trial of everolimus was 4.5 mg/m^2 daily, which resulted in excellent efficacy and minimal toxicity.[42]

Everolimus displays active Bcrp1 and P-gp inhibition, which has been shown to improve brain parenchymal penetration of TKIs.[24] Additionally, mTOR is downstream of the RTK/PI3K/AKT signaling pathway, which is active in pediatric gliomas with growth factor receptor activation, thus providing an additional benefit to its use in the treatment of these tumors.[7,24]

3.6 Novel imaging biomarkers for therapy response:

Conventional gadolinium (Gd) contrast-enhanced T1-weighted (T1W) and T2W or fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI) may inadequately represent the extent of actively proliferating human high-grade glioma.[43,44] Differentiating hypercellular components of high-grade glioma from vascular components, edema, and normal tissue is a challenge using FLAIR and conventional diffusion-weighted imaging (DWI) when standard b-values (1000 s/mm^2 or lower) are used for diffusion measurement.[43] Increasing b-values in the diffusion sequence beyond the conventional b-values has been shown to distinctly reveal high cellularity tumor volume (HCV).[43] We propose to study the utility of adding increased b-value (3000 s/mm^2) DWI to surveillance in the measurement of response to therapy. Both of these imaging correlates can be performed at tertiary clinical radiology centers. These imaging biomarkers will be explored as secondary objectives, and will be examined separately from primary imaging endpoints.

3.7 Prospective analysis of CSF and plasma cell-free tumor DNA as a marker for therapy response:

Due to their location in midline or deep structures, pediatric brain tumors would greatly benefit from non-invasive methods for tumor diagnosis and treatment surveillance. Recently, digital polymerase chain reaction–based technologies have been developed to evaluate the ability of cell-free tumor DNA to detect various cancer types.[45] Cell-free DNA shed by cancer cells has been shown to provide tumor specific genomic information. Dr. Bettwegowda (Johns Hopkins), co-investigator for this study, has shown that cerebrospinal fluid tumor DNA (CSF-tDNA) can be

used as a patient-specific genomic biomarker in three-fourths of primary adult brain and spinal cord tumors.[46]

We propose to prospectively evaluate CSF and plasma tDNA as a marker of disease activity in patients undergoing therapy. We will measure and compare CSF and plasma samples prior to cycle 1 and 3 (mandatory) and with surveillance MRIs thereafter (optional). Dr. Koschmann will work with Dr. Bettwegowda (Johns Hopkins) to use PCR-based ultra-deep sequencing platform to perform focused ultra-deep sequencing of confirmed tumor DNA alterations to assess the ability to use this assay to provide minimal residual disease estimates and to predict treatment response and recurrence (no clinical treatment decisions will be made based on these correlate tests). Dr. Koschmann (University of Michigan) will use genomic information from sequenced tumors to develop a non-invasive pediatric glioma CSF sequencing panel for validation on CSF samples.

3.8 Proposed Study

This trial will evaluate the activity of dasatinib in combination with everolimus for children with gliomas harboring *PDGFR* or alterations, including newly diagnosed high-grade (grade III-IV) glioma after radiation (stratum A) and recurrent/progressive grade II-IV glioma (stratum B).

Participants must have a genomic (DNA and/or RNA) alteration (mutation, fusion, and/or amplification) involving *PDGF-A*, *PDGF-B*, *PDGFR-A*, *PDGFR-B*, as identified by tumor sequencing. Patients who undergo sequencing at the University of Michigan to determine eligibility will first enroll in the U of Michigan Mi-ONCOSEQ study (HUM00056496). MI-ONCOSEQ includes clinically integrated exome (tumor and germline DNA) and transcriptome (tumor RNA) sequencing (CLIA certified), as previously described.[11,17,47] Off-site (non U of Michigan academic or private) CLIA-certified tumor DNA/RNA sequencing can also be used to determine eligibility.

Patients will receive oral dasatinib and everolimus daily. The planned starting dose for dasatinib is 60 mg/m^2 twice daily as oral pill (as oral suspension if the patient is unable to swallow pills). The maximum dose of dasatinib is 100 mg twice daily if < 18 years old, and 140mg once daily if ≥ 18 years old. No dose escalation is planned. The planned starting dose for everolimus (oral pill or /Affinitor disperz tablet) is 3.0 mg/m^2 once daily, with titration of dosing to keep everolimus trough level of 5-15 ng/ml. The maximum dose of everolimus is 10mg once daily. Both agents will be taken daily for 28 days. This is considered 1 cycle. CSF and plasma dasatinib pharmacokinetics will be performed by the University of Michigan to determine whether blood-CSF permeability of dasatinib is impacted by dual therapy with everolimus. Prior to first cycle only, dasatinib will be taken as mono-therapy for 1 week followed by CSF collection in order to establish mono-therapy dasatinib CSF level. Cycles will be repeated every 28 days and patients may receive up to 24 cycles. Up to 32 participants will be enrolled on this trial.

4.0 Eligibility Requirements

Eligibility requirements 4.2 (disease status) and 4.4 (consent) must be met prior to tumor testing. All other eligibility requirements must be met prior to the start of treatment.

4.1 Patient

4.1.1 Age at enrollment: Greater than 1 year and less than 50 years

4.1.2 BSA (body surface area): BSA greater than 0.3 m2

4.1.3 Functional status: Karnofsky > 50% for patients > 16 years of age and Lansky > 50% for patients < 16 years of age. Neurologic deficits in patients with CNS tumors must have been relatively stable for a minimum of 7 days. Patients who are unable to walk because of paralysis, but who are able to sit in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

4.1.4 Organ function:

4.1.4.1 Adequate bone marrow function including
- ANC > 750
- Platelet count > 100,000/uL without platelet transfusion within the past 7 days

4.1.4.2 Adequate liver function defined as Bilirubin < 1.5 x upper limit of normal and ALT < 2.5 x upper limit of normal.

4.1.4.3 Adequate renal and metabolic function defined as:

a. Creatinine clearance or radioisotope glomerular filtration rate (GFR) >70 mL/min/1.73 m^2, or

b. A serum creatinine based on age/gender as follows:

Age	Maximum Serum Creatinine (mg/dL)	
	Male	**Female**
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4
The threshold creatinine values in this table were derived from the Schwartz formula for estimating GFR utilizing child length and stature data published by the CDC.		

c. Urine protein:creatinine (UPC) ratio of <1; or a urinalysis that is negative for protein; or 24-hour urine protein level < 1000 mg/dL

4.1.5 Patients with known seizure disorder must have seizures adequately controlled with non-enzyme inducing antiepileptic medications

4.1.6 No increase in steroid dose within the past 7 days.

4.2 Disease

4.2.1 STRATUM A: histological confirmation of a newly diagnosed high-grade glioma or DIPG

4.2.2 STRATUM B: histological confirmation (at diagnosis or relapse) of a recurrent or progressive grade II-IV glioma (including DIPG)

4.2.3 ALL STRATA:

4.2.3.1 Location: Primary brain or spine tumor are eligible, including tumors with metastases, multiple lesions

4.3 Prior therapies

4.3.1 Patients must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy.

4.3.2 Myelosuppressive chemotherapy: Must not have received within 3 weeks (6 weeks if prior nitrosourea).

4.3.3 Hematopoietic growth factors: At least 7 days since the completion of therapy with a growth factor, 14 days for long- acting (e.g. PEG-filgrastim)

4.3.4 Biologic (anti-neoplastic agent): At least 7 days or 3 half-lives (whichever is longer) since the completion of therapy with a biologic agent.

4.3.5 Radiation therapy:

- Strata A: ≥ 2 weeks and </= to 12 weeks must have elapsed from radiation.
- Strata B: ≥ 2 weeks must have elapsed from focal radiation.

4.3.6 Surgery: > 3 weeks from major surgery. If recent craniotomy, adequate wound healing must be determined by neurosurgical team.

4.3.7 Autologous Stem Cell Transplant or Rescue: No evidence of active graft vs. host disease and ≥ 4 weeks must have elapsed.

4.4 Consent

All patients and/or a legal guardian must sign institutionally approved written informed consent and assent documents.

4.5 Tumor Testing

PDGF or alteration: Participants must have a genomic (DNA and/or RNA) alteration (mutation, fusion, and/or amplification) involving *PDGF-A*, *PDGF-B*, *PDGFR-A*, *PDGFR-B*, or, as

identified by tumor (FFPE or fresh, diagnosis or relapse tissue, but relapse tissue preferred) sequencing. Sequencing will be performed through the University of Michigan MI-ONCOSEQ study (CLIA-certified), or other (non-U of Michigan) CLIA-certified tumor DNA or RNA sequencing. For MI-ONCOSEQ, at least 1 mm cubed of tumor tissue or >1 core biopsy of tumor tissue is required, fresh or FFPE].

4.6 Exclusion criteria

4.6.1 Reproductive Exclusions: Patients who are breastfeeding, pregnant or refuse to use an effective form of birth control are excluded. Abstinence is considered an effective form of birth control.

4.6.2 Infection: Patients with uncontrolled infection are excluded.

4.6.3 Other medications:

4.6.3.1 Patients receiving other anti-neoplastic agents are excluded.

4.6.3.2 Patients requiring strong CYP3A4 or PGP inhibitors are excluded (Appendix B).

4.6.3.3 Patients requiring anticoagulation or with uncontrolled bleeding are excluded.

4.6.3.4 Patients on steroids for symptom management must be on a stable dose for 7 days prior to start of treatment.

4.6.4 Allogeneic stem cell transplant: Patients within 1 year of allogeneic stem cell transplant, patients with active GVHD or requiring immunosuppression are excluded.

4.6.5 Previous hypersensitivity to rapamycin or rapamycin derivatives.

5.0 Treatment/Intervention Plan

Participants will be evaluated for toxicity using CTCAE v4. Stopping rules for toxicity and response are included below. Patients will receive oral dasatinib and everolimus daily. The maximum dose of dasatinib is 100 mg twice daily if < 18 years old, and 140mg once daily if $\geq$ 18 years old. No dose escalation is planned. The planned starting dose for everolimus (oral pill or /Affinitor disperz tablet) is 3.0 mg/m^2 once daily, with titration of dosing to keep everolimus trough level of 5-15 ng/ml. The maximum dose of everolimus is 10mg once daily. Doses of both agents may be rounded to the nearest tablet size within 20% of calculated dose. Both agents will be taken daily for 28 days. This is considered 1 cycle. Cycles will be repeated every 28 days and patients may receive up to 24 cycles. Up to 32 participants will be enrolled on this trial.

CSF and plasma dasatinib pharmacokinetics will be performed by the University of Michigan to determine whether blood-CSF permeability of dasatinib is impacted by dual therapy with everolimus. Prior to first cycle, dasatinib will be taken as mono-therapy for 1 week followed by CSF collection in order to establish mono-therapy dasatinib CSF level. After cycle 2, CSF will be collected to establish dual-therapy dasatinib level (while on everolimus). Additionally, CSF collected at these time points will be used to assess CSF cell-free tumor DNA burden as a possible predictor of treatment response. CSF collection at subsequent prior to subsequent odd-numbered cycles for CSF cell-free tumor DNA analysis will be optional.

5.1 Therapy

5.1.1 Dasatinib will be administered orally at a dose of 60 mg/m^2 orally, twice daily days 1-28 each cycle continuously. The maximum dose of dasatinib is 100 mg twice daily if < 18 years old, and 140mg once daily if ≥ 18 years old. Ideally, capsules should be swallowed whole with a glass of water. However, if patient is unable to swallow capsules, a suspension form may be prepared for administration. Medication should be taken at least one hour before and two hours after large meals.

5.1.2 Everolimus will be administered orally at a dose of 3.0 mg/m^2 daily days 1-28 each cycle continuously. The maximum dose of everolimus is 10mg once daily. Ideally, tablets should be swallowed whole with a glass of water. If patients are unable to swallow tablets, a suspension form may be prepared for administration (same dose). The medication should be taken at the same time every day and either consistently with food or consistently without food.

5.1.3 Each cycle will be repeated every 28 days and when the following criteria are met:

5.1.3.1 Absolute neutrophil count must be greater than 750/uL

5.1.3.2 Platelet count must be greater than 75,000/uL

5.1.3.3 Bilirubin < 1.5 x upper limit of normal and ALT must be <2.5 x upper limit of normal

5.2 Supportive care

5.2.1 Neutropenic precautions: Patients and/or parents or guardians should receive education about the risk of infection and neutropenia. Patients should receive laboratory evaluation for neutropenia at the time of fever or expected infection. Empiric IV antibiotic therapy will be given for patients found to have fever and neutropenia according to institutional guidelines.

5.2.2 Infection prophylaxis: Patients should receive appropriate prophylaxis for pneumocystis (PCP) pneumonia starting during first cycle and continuing until a minimum of three months after completion of chemotherapy.

5.2.3 Transfusion of blood products: Blood products should be transfused for bleeding or symptomatic anemia. In asymptomatic patients, transfusion should be used to maintain platelet count above 30,000 and hemoglobin above 7 g/dl. Patients cannot be transfused within 7 days of study start (Section 4.1.4.1). Platelet could must be > 75,000 to start a new cycle (Section 5.1.3.1).

5.2.4 Antiemetic management: Appropriate anti-emetics may be used prior to chemotherapy. Steroids are discouraged for antiemetic management.

5.2.5 Cytokine support: Patients should not receive routine cytokine support. In the setting of neutropenia and documented life- threatening infection, cytokine support and/or granulocyte infusion should be considered.

5.2.6 Electrolyte and fluid support: Diarrhea, dehydration and electrolyte abnormalities are known potential side-effects of dasatinib. Additional oral or intravenous fluid, sodium, calcium, magnesium, phosphorus or potassium should be given to maintain euvolemia and electrolyte levels

within normal range for patients who develop diarrhea to prevent dehydration or symptomatic electrolyte abnormalities. Imodium may be used to manage symptomatic diarrhea.

5.3 Therapy Modifications for Toxicity

All dose modifications should be based on the worst preceding toxicity. The severity of adverse events will be graded utilizing the National Cancer Institute (NCI) CTCAE, version 4.

5.3.1 Definition of Dose Limiting Toxicity (DLT)

DLT will be defined as any of the following events that are at least possibly, probably, or definitely attributable to dasatinib or everolimus. See below for the potential dasatinib and everolimus specific side effects. Dose-limiting hematological and non-hematological toxicities are defined differently.

5.3.1.1 Hematological Dose-Limiting Toxicity

- Grade 4 thrombocytopenia (platelet count < 25,000/mm3) or Grade 4 neutropenia
- Any hematologic toxicity requiring dose reduction or treatment interruption for >14 days
- ≥ Grade 2 arterial thromboembolic events (visceral arterial ischemia, peripheral ischemia, or ischemia cerebrovascular)
- Grade 3 or 4 venous thromboembolic event
- Any thrombotic event (other than central venous line [CVL] associated thrombosis) requiring systemic anticoagulation

5.3.1.2 Non-Hematological Dose-Limiting Toxicity

- Any Grade 4 non-hematologic toxicity, with the exception of alopecia
- Any Grade 3 non-hematological toxicity, with the specific exclusion of the following: Grade 3 nausea and vomiting of < 3 days duration; Grade 3 fever or infection, Grade 3 hypophosphatemia, hypokalemia, hypocalcemia or hypomagnesemia responsive to oral supplementation within 7 days of start of supplement
- Grade 2 allergic reactions that necessitate discontinuation of protocol therapy will not be considered a dose-limiting toxicity
- Any Grade 2 non-hematological toxicity that persists for ≥ 7 days and is considered sufficiently medically significant or sufficiently intolerable by patients that it requires treatment interruption may also be considered a DLT following discussion with the Study chair and an appropriate pharmaceutical medical monitor.
- Hypertension will be graded according to the NCI CTCAE; however, dose limiting hypertension will be considered as the following:
    - Grade 4 hypertension
    - A blood pressure >25 mmHg above the 95th percentile for age, height, and gender confirmed by repeated measurement on the same day
    - In patients already on anti-hypertensive therapy due to previous hypertension, any blood pressure 1-25 mmHg above the 95th percentile for age, height, and gender for > 14 days

5.3.1.3 Dose Modifications for Hematologic Toxicity

All dose modifications should be based on the worst preceding toxicity. For step-wise dose reductions, see section 5.3.4 for the dosing nomogram. For all patients:

- If Grade 4 neutropenia occurs, dasatinib should be held, and CBC should be checked at least twice a week (every 3 to 4 days) until ANC ≥ 750/μL. Subsequent doses of dasatinib should be given at a reduced dose, when ANC ≥ 750/μL
- If Grade 4 thrombocytopenia occurs, dasatinib should be held. CBCs should be checked at least twice a week (every 3 to 4 days) until platelet count ≥ 75,000/μL without need for platelet transfusion in the preceding 7 days. Subsequent doses of dasatinib should be given at a reduced dose, when platelets count ≥ 75,000/μL without need for platelet transfusion in the preceding 7 days.
- For patients who have a dose limiting hematological toxicity that does not resolve to the parameters defined above within 14 days of holding dasatinib: if re-challenge is to be considered the Study Chair must be contacted and provide approval prior to re-challenge.

5.3.1.4 Dose Modifications for Non-Hematological Toxicity

For any non-hematologic dose limiting special toxicity attributed to dasatinib or everolimus, the respective agent should be held. If the non-hematologic DLT returns to baseline within 14 days of holding dasatinib, subsequent doses of reduced agent should be given at a reduced dose. If toxicity does not resolve to meet continued treatment parameters within 14 days of drug discontinuation, and if re-challenge is to be considered, the Study Chair must be contacted to discuss. If DLT recurs in a patient who has resumed treatment after the maximum allowed dose reductions, the patient must be removed from protocol therapy, unless the patient has been approved for a further dose reduction following discussion with the Study Chair.

5.3.2 Dasatinib

5.3.2.1 Special Toxicities Potentially Related to Dasatinib:

5.3.2.1.1 Pleural effusion

Effusions are a documented side effect of dasatinib. If symptomatic grade 2 pleural effusions develop for > 1 week or grade 3, dose should be held and then restarted at reduced dose when symptoms resolve.

5.3.2.1.2 Diarrhea

Diarrhea is a common side effect of dasatinib. If diarrhea is not adequately controlled to less than grade 3 with supportive care including hydration and loperamide, then dose may be held until symptoms resolve and restarted at the reduced dose

5.3.2.1.3 Electrolyte Abnormalities

Serum K^+, Ca^{++}, Mg^{++}, phosphate levels should be monitored given the risk of prolonged QTc with dasatinib. If serum Ca^{++} is abnormal, ionized calcium should be obtained and interventions based on ionized calcium only. Dasatinib should be held and an EKG obtained for ≥ Grade 2

hypokalemia or hyperkalemia; and ≥ Grade 3 Ca^{++}, Mg^{++}, phosphate abnormalities. Dasatinib may be resumed at a reduced dose when K^{+} abnormality is ≤ Grade 1 or within institutional limits, and other abnormalities (Ca^{++}, Mg^{++}, phosphate) are ≤ Grade 2.

5.3.2.1.4 QT Prolongation:

If QTc elevation is seen, the following adjustments should be made:

- QTc interval < 500 msec: No specific therapy needed. Continue dasatinib at the same dose. Serum electrolytes should be monitored and repleted if low.
- QTc interval ≥ 500 msec: Hold dasatinib, review concomitant medications, and replete serum electrolytes as needed. The EKG should be repeated within 7 days and if the QTc interval is < 500 msec then resume dasatinib at a reduced dose. If the QTc interval remains ≥ 500 msec, continue obtain EKG in 7 days.

5.3.2.1.5 Other toxicity

For any grade 3 toxicity determined to be clinically significant and at least possibly related to dasatinib treatment, drug should be held until toxicity resolves to <grade 3, and dose reduction should be made as described below, with the specific exception of nausea, vomiting or electrolyte imbalances which are corrected by supplementation.

5.3.3 Everolimus

5.3.3.1 Special Toxicities Potentially Related to Everolimus

5.3.3.1.1 Mucositis

Mucositis and oral ulceration is a common/known side effect of everolimus. If grade 3 mucositis occurs on therapy, everolimus should be held and then restarted at the reduced dose (see chart below).

5.3.3.1.2 Thrombosis

If thrombosis occurs, the following adjustments to everolimus should be made:

	Dose Modifications & Evaluations
Grade 2	Continue everolimus at the current dose and monitor as clinically indicated.
Grade 3 (CVL associated only)	1. Hold everolimus 2. Treat thrombosis according to institutional standards; would consider removal of the CVL. 3. Resume everolimus when all symptoms have resolved. If anticoagulation is required, use with caution.

All non-CVL associated Grade 3 AND	Discontinue everolimus permanently and remove from protocol therapy

5.3.4 Dose reductions for specific agents if dose reduction warranted after dose held.

	Starting dose	1st dose reduction	2nd dose reduction
Dasatinib	60 mg/m^2/ dose BID	60 mg/m^2/ dose daily	30 mg/m^2/ dose daily
Everolimus	3 mg/m^2/ dose daily	2 mg/m^2/dose daily	1 mg/m^2/ dose daily

Additionally, there will be stopping rules for toxicity to avoid excessive toxicity. If more than one patient develops clinically relevant grade 3 or greater toxicity possibly or probably attributed to study treatment, then subsequent patients should start at the first dose reduction level of that agent. If an additional patient on the same treatment regimen develops clinically relevant grade 3 or 4 toxicity possibly or probably attributed to study treatment, then the study will be placed on hold for further patient enrollment until further review.

6.0 Biology and Imaging Correlates

6.1 Mandatory biology testing

Participants must have a genomic (DNA and/or RNA) alteration (mutation, fusion, and/or amplification) involving *PDGF-A*, *PDGF-B*, *PDGFR-A*, *PDGFR-B*, or, as identified by tumor (FFPE or fresh, diagnosis or relapse tissue, but relapse tissue preferred) sequencing. Sequencing will be performed through the University of Michigan MI- ONCOSEQ study (CLIA-certified, HUM00056496), or other (non-U of Michigan) CLIA-certified tumor DNA or RNA sequencing. For MI-ONCOSEQ, at least 1 mm cubed of tumor tissue or >1 core biopsy of tumor tissue is required, fresh or FFPE].

CSF and plasma dasatinib pharmacokinetics will be performed by the University of Michigan to determine whether blood-CSF permeability of dasatinib is impacted by dual therapy with everolimus.

6.1.1 Tumor sequencing (mandatory): To confirm eligibility, participants must have a genomic alteration (mutation, fusion, or amplification involving *PDGF-A*, *PDGF-B*, *PDGFR-A*, *PDGFR-B*. Participants will undergo tumor (FFPE or fresh, diagnosis or relapse tissue, but relapse tissue preferred) sequencing to determine eligibility. CLIA certified DNA or RNA sequencing that identifies an alteration in *PDGF-A*, *PDGF-B*, *PDGFR-A*, *PDGFR- B*, will be accepted. Over-expression (RNA) alone is not sufficient, and must be accompanied by DNA alteration or DNA/RNA fusion.

Patients will be offered enrollment/ tumor/germline sequencing through the U of Michigan Mi-Oncoseq protocol. Enrollment on Mi-Oncoseq will be performed through separate IRB approved consent form and enrollment process. Mi-Oncoseq enrollment is not necessary for enrollment on

this trial, and alternate CLIA-certified sequencing will be accepted to confirm eligibility. Mi-Oncoseq clinically integrated sequencing will be performed according to previous published methodology.[11,17,47]

6.1.2 Dasatinib CSF/plasma pharmacokinetics (mandatory): CSF and plasma dasatinib pharmacokinetics will be performed by the University of Michigan to determine whether blood- CSF permeability of dasatinib is impacted by dual therapy with everolimus. Prior to first cycle, dasatinib will be taken as mono-therapy for 1 week followed by CSF collection in order to establish mono-therapy dasatinib CSF level. After cycle 2, CSF will be collected to establish dual-therapy dasatinib level (while on everolimus).

6.1.3 CSF and plasma cell-free tumor DNA analysis: CSF collected at above time points (prior to first and third cycle) will be used to assess CSF cell-free tumor DNA to explore tumor DNA burden as a possible predictor of treatment response.

6.1.4 Everolimus serum level of everolimus: Everolimus serum level will be monitored monthly throughout therapy at clinical lab to adjust dosing to trough concentration to 5-15 ng/ml.

6.2 Optional Biology Testing

Additional optional correlate assays include CSF and plasma collection for CSF tDNA analysis at subsequent odd-numbered cycles.

6.3 Mandatory Imaging Correlates

Disease evaluation by MRI will be performed every two cycles. High b-value diffusion weighted imaging (DWI) will be performed at the time of clinical MRIs to evaluate their utility as imaging biomarkers for therapy response.

Correlative Objective (Name of Correlate & Lead PI and Site)	**Imaging Technique**	**Organ(s) Scanned and Timing of Scans**	**M/O**
MRI brain	Magnetic Resonance Imaging	Brain (+/- Spine); Baseline and with interval scans (every second cycle)	M
Hypercellularity by High b-Value Diffusion-Weighted Imaging as an Imaging Biomarker for Dasatinib Therapy Response (Hemant Parmar, M.D./Yue Cao, PhD; University of Michigan)	Diffusion-Weighted Imaging	Brain; Baseline and with interval scans (every second cycle)	M

Diffusion-Weighted Imaging: In addition to conventional clinical post-Gd T1WI and FLAIR- MRI, DWI in 3 orthogonal directions with b values of 0, 1000, and 3000 s/mm2 will be acquired in all patients. DWI will be acquired with the following imaging protocol.

2D multiple b-value diffusion weighted images

Sequence type	Ep2d (*ep_b0)	**TE (ms)**	Min(98)
3D or 2D	2D	**TR (ms)**	8200

FOV (mm)	270x270x144	**Diffusion Gradient**	Bipolar*
Voxel Size (mm)	1.4x1.4x4.8	**3 orthogonal diffusion directions**	yes
Slice thickness and gap	4mm and 20%	**b-value (s/mm2)**	0, 1000, 2000 and 3000
Orientation	Axial	**Average**	1, 2, 3 and 4 for 4 b-values
# of slices	Whole brain	**Parallel imaging factor**	4**

*: to reduce eddy current;
**: to reduce geometric distortion
***: We will test if we can replace this sequence by the RESOLVE sequence to further improve geometric accuracy. We assessed the geometric distortion using the current protocol and reported in reference 3.
Additional questions and guidance on DWI acquisition should be addressed to Yue Cao, PhD (yuecao@med.umich.edu).

7.0 Evaluations to Be Obtained

7.1 Pre-Treatment Evaluation

MRI must be obtained within 28 days prior to starting therapy. If patient undergoes surgery, MRIs are required pre-surgery, and within 72 hours post-surgery. Timing of other evaluations is indicated below. Any child who receives two cycles of therapy and undergoes MRI prior to third cycle will be considered evaluable.

7.2 On-Study Evaluation

Studies to be Obtained	Pre-Study		Prior to Cycle 1	During Cycle 1		Prior to Every Cycle[T1]	Prior to Every Other Subsequent Cycle [T1]	Prior to 3rd Cycle[T1]	End of therapy[T2]
	-14 to 0	-7 to 0	Day -6	Day 1	Day 14				
History, Physical Exam, Vitals[T3]		X	X	X	X	X			X
Height, weight, BSA[T4]		X	X	X		X			
Performance status		X	X	X		X			X
Concomitant Medicines		X		X	X	X			X
CBC, differential, platelets		X	X	X	X[T5,T6]	X			X
Adverse Event Collection		X		X	X	X			X
Electrolytes, including K, Ca, Phos and Mg, ALT and bilirubin		X	X	X	X	X			X
Disease evaluation (MRI brain including high b-value DWI)[T7]	X						X[T8]	X[T8]	X
FFPE or fresh tissue for sequencing	X								
Serum pregnancy test[T9]		X		X		X			
EKG, including QTc[T10]	X						X	X	
Dasatinib therapy alone			X[T11]						
Dasatinib CSF pharmacokinetics[T12]				X			X	X	
Dasatinib plasma level				X				X	
Everolimus serum level				X		X			
Cell free tumor DNA-CSF/blood tDNA (mandatory)				X				X	
Cell free tumor DNA-CSF/blood tDNA (optional)							X		

[T1] Window for assessment is Day 1 +/- 3 days for any assessment with the exception of disease evaluations.

[T2] Should be performed, if possible, at the time the patient comes off protocol therapy regardless of the reason, unless the test or procedure has been performed within the past 2 weeks.

[T3] Blood pressures will be measured with an appropriate sized cuff at rest. Blood pressure measurement will be repeated within the same day if the blood pressure (BP) is elevated (> the 95th percentile for age, height, and gender). If both BP measurements are > 95th percentile for age, height, and gender, follow the guidelines for elevated BP with dasatinib.
[T4] Evaluations obtained for screening will ONLY be repeated at Cycle 1 Day 1 if they are older than 7 days.
[T5] If patients have Grade 4 neutropenia, then CBCs should be checked every 3 to 4 days until recovery to Grade ≤3.
[T6] If patients have Grade 3 or 4 thrombocytopenia, then CBCs should be checked twice a week until recovery to Grade ≤2.
[T7] Obtain spine MRI with brain MRI if there is spinal disease involvement at enrollment. MRI must be obtained within 90 days prior to starting therapy. If patient undergoes surgery, MRIs are required pre-surgery, and within 72 hours post-surgery.
[T8] Disease evaluation should be performed on Day 1 of the current cycle -7 days (Day 21-28 of the previous cycle) prior to Cycles 3 and every other cycle thereafter. If the institutional investigator determines that the patient has progressed based on clinical or laboratory evidence, the investigator can remove the patient from protocol therapy, he/she may opt not to confirm this finding radiographically; however, an end of study disease assessment should be completed.
[T9] Females of childbearing potential require a negative serum pregnancy test prior to starting treatment. Patients of childbearing potential must use an acceptable method of birth control. Abstinence is an acceptable method of birth control.
[T10] More frequent EKG monitoring is required for Grade 2 potassium and Grade 3 calcium (confirmed by ionized calcium), magnesium and phosphorous abnormalities. See section on electrolyte abnormalities with dasatinib
[T11] Dasatinib therapy will be administered alone 7 days prior to Cycle 1.
[T12] Dasatinib CSF pharmacokinetics will be required prior to the first cycle and after cycle 2. CSF analysis at subsequent odd numbered cycles will be optional.

7.3 Follow-up Evaluation

Survival endpoints will be estimated including time to progression as well as progression-free and overall survival rates at the 6 month, 1 year and 2 year time point (+/- 2 months) from start of study treatment. Follow-up will be accomplished by standard of care visits and MRI if at U of M, or a phone call to the patient at above time points if patient is primarily receiving care elsewhere. Patients will be contact at one and 2 year interval to confirm MRI and visit is scheduled (if receiving care elsewhere).

8.0 Therapeutic Agents

8.1 Use of Commercial Medications

The products used in this study are the commercially available US-Licensed product without modification to the approved packaging.

8.2 Toxicities/Side Effects

The following are known/expected side effects of agents used on this study

8.2.1 Dasatinib

Frequency defined below:

- Cardiovascular: Facial edema, peripheral edema (~10%); Cardiac conduction disturbance (7%), ischemic heart disease (4%), cardiac disease (≤4%; includes cardiac failure, cardiomyopathy, diastolic dysfunction, ejection fraction decreased, left ventricular dysfunction, ventricular failure), edema (≤4%; generalized), pericardial effusion (≤4%; grades 3/4: ≤1%), prolonged Q-T interval on ECG (≤1%), cardiac arrhythmia, chest pain, flushing, hypertension, palpitations, tachycardia
- Central nervous system: Headache (12% to 33%), fatigue (8% to 26%), pain (11%); Chills, depression, dizziness, drowsiness, insomnia, myasthenia, neuropathy, peripheral neuropathy (all < 10%)
- Dermatologic: Skin rash (11% to 21%; includes drug eruption, erythema, erythema multiforme, erythematous rash, erythrosis, exfoliative rash, follicular rash, heat rash, macular rash, maculopapular rash, milia, papular rash, pruritic rash, pustular rash, skin exfoliation, skin irritation, urticaria vesiculosa, vesicular rash), pruritus (12%); Acne vulgaris, alopecia, dermatitis, eczema, hyperhidrosis, urticaria, xeroderma (all < 10%)
- Endocrine & metabolic: Fluid retention (19% to 48%; grades 3/4: 1% to 8%; cardiac-related: 9%); Hyperuricemia, weight gain, weight loss (all < 10%)
- Gastrointestinal: Diarrhea (17% to 31%), nausea (8% to 24%), vomiting (5% to 16%), abdominal pain (7% to 12%); Constipation (10%), gastrointestinal hemorrhage (2% to 9%; grades 3/4: 1% to 7%), abdominal distention, change in appetite, colitis (including neutropenic colitis), dysgeusia, dyspepsia, enterocolitis, gastritis, mucositis, stomatitis (all less <10%); Increased serum bilirubin (grades 3/4: ≤6%), increased serum ALT (grades 3/4: ≤5%), increased serum AST (grades 3/4: ≤4%), ascites (≤1%)
- Hematologic & oncologic: Thrombocytopenia (grades 3/4: 22% to 85%), neutropenia (grades 3/4: 29% to 79%), anemia (grades 3/4: 13% to 74%), hemorrhage (8% to 26%; grades 3/4: 1% to 9%), febrile neutropenia (4% to 12%; grades 3/4: 4% to 12%); Intracranial hemorrhage (≤3%; grades 3/4: ≤3%), bruise (<10%)
- Infection: Infection (9% to 14%; includes bacterial, fungal, viral); Herpes virus infection, sepsis (<10%)
- Local: Localized edema (3% to 22%; grades 3/4: ≤1%; superficial)
- Neuromuscular & skeletal: Musculoskeletal pain (<22%), myalgia (7% to 13%), arthralgia (≤13%); Muscle spasm (5%), stiffness, weakness (<10%)
- Renal: Increased serum creatinine (grades 3/4: ≤8%)

- Respiratory: Pleural effusion (5% to 28%; grades 3/4: ≤7%), dyspnea (3% to 24%); Pulmonary hypertension (≤5%; grades 3/4: ≤1%), pulmonary edema (≤4%; grades 3/4: ≤3%); cough, pneumonia (bacterial, viral, or fungal), pneumonitis, pulmonary infiltrates, upper respiratory tract infection (all < 10%)
- Miscellaneous: Fever (6% to 18%)

8.2.2 Everolimus

Common:

- Cardiovascular: Hypertension (kidney transplant recipients, 30% ; advanced renal cancer, 4%; subependymal giant cell astrocytoma, 4%), Peripheral edema (kidney transplant recipients, 45% ; advanced renal cancer, 25%; subependymal giant cell astrocytoma, 4%)
- Dermatologic: Infection of skin AND/OR subcutaneous tissue (18%), Rash (advanced renal cancer, 29%; subependymal giant cell astrocytoma, 18%; advanced pancreatic neuroendocrine tumor, 59%)
- Endocrine metabolic: Decreased phosphate level (37% to 40%), Dyslipidemia (15%), Hyperlipidemia (21%), Increased glucose level, All grades (advanced pancreatic neuroendocrine tumor, 75%; advanced renal cell cancer, 57%; subependymal giant cell astrocytoma, 25%), Serum cholesterol raised (advanced renal cancer, 77%; subependymal giant cell astrocytoma, 68% ; advanced pancreatic neuroendocrine tumor, 66%), Serum triglycerides raised (advanced renal cell cancer, 73%; subependymal giant cell astrocytoma, 43% ; advanced pancreatic neuroendocrine tumor, 39%)
- Gastrointestinal: Constipation (kidney transplant recipients, 38% ; subependymal giant cell astrocytoma, 11%), Diarrhea (kidney transplant recipients, 19% ; advanced renal cancer, 30%; subependymal giant cell astrocytoma, 25%; advanced pancreatic neuroendocrine tumor, 50%), Loss of appetite (25% to 30%), Nausea (26% to 29%), Oropharyngeal mucositis, Stomatitis (kidney transplant recipients, 8% ; advanced renal cancer, 44%; advanced pancreatic neuroendocrine tumor, 70%; subependymal giant cell astrocytoma, 86%), Ulcer of mouth, Vomiting (advanced renal cancer, 20%; subependymal giant cell astrocytoma, 21%; advanced pancreatic neuroendocrine tumor, 29% ; kidney transplant recipients, 15%)
- Hematologic: Anemia (kidney transplant recipients, 26% ; renal cell cancer, 50% or higher), Decreased hemoglobin, All grades (advanced renal cancer, 92%; subependymal giant cell astrocytoma, 39% ; advanced pancreatic neuroendocrine tumor, 86%), Decreased lymphocyte count, All grades (45% to 51%), Decreased platelet count, All grades (21% to 45%)
- Hepatic: ALT/SGPT level raised (advanced renal cell cancer, 21%; subependymal giant cell astrocytoma, 46%; advanced pancreatic neuroendocrine tumor, 48%), AST/SGOT level raised (advanced renal cancer, 25%; advanced pancreatic neuroendocrine tumor, 56%; subependymal giant cell astrocytoma, 89%)
- Immunologic: Surgical wound finding (35%)
- Neurologic: Asthenia (33%)
- Otic: Otitis media (36%)

- Renal: Serum creatinine raised (advanced renal cancer, 50%; advanced pancreatic neuroendocrine tumor, 19%; subependymal giant cell astrocytoma, 11% ; kidney transplant recipients, 18%), Urinary tract infectious disease (advanced pancreatic neuroendocrine tumor, 16% ; kidney transplant recipients, 22%)
- Respiratory: Cough (advanced renal cancer, 30%; advanced pancreatic neuroendocrine tumor, 25%; subependymal giant cell astrocytoma, 21% ; kidney transplant recipients, 7%), Dyspnea (20% to 24%), Sinusitis (39%), Upper respiratory infection (kidney transplant recipients, 16% ; subependymal giant cell astrocytoma, 82%)
- Other: Fatigue (advanced pancreatic neuroendocrine tumor, 45% advanced renal cancer, 31%; subependymal giant cell astrocytoma, 7% kidney transplant recipients, 9%), Fever (advanced renal cancer, 20%; advanced pancreatic neuroendocrine tumor, 31%; subependymal giant cell astrocytoma, 32% ; kidney transplant recipients, 19%)

Serious:

- Hematologic: Decreased hemoglobin, Grade 4 (advanced renal cancer, 1% ; advanced pancreatic neuroendocrine tumor, 15%), Decreased lymphocyte count, Grade 4 (advanced renal cancer, 2% ; advanced pancreatic neuroendocrine tumor, 16%), Hemorrhage (3%), Leukopenia (kidney transplant recipients, 3% ; subependymal giant cell astrocytoma, 54% ; advanced pancreatic neuroendocrine tumor, 43%), Thrombosis, Thrombotic microangiopathy, Thrombotic thrombocytopenic purpura
- Immunologic: Infectious disease (advanced renal cancer, 37% ; kidney transplant recipients, 62%)
- Neurologic: Seizure (29%)
- Renal: Hemolytic uremic syndrome, Renal failure (3%), Thrombosis of renal artery (1% to less than 10%)
- Respiratory: Non-infectious pneumonia (14% to 17%), Pleural effusion (7%), Pneumonia, Pulmonary embolism

9.0 Criteria for Therapeutic Response/Outcome Assessment

Although this is not a cooperative group study, for the purpose of uniformity and in order to compare results of this study to other contemporary studies, response assessment will be performed according to Children's Oncology Group guidelines.[48] Response will be assessed using MRI and two-dimensional measurement of target lesions.

9.1 Methodology to Determine Tumor Measurement

Tumor response criteria are determined by changes in size using all 3 dimensional measurements: width (W), transverse (T), and length (L) measurements. Thus for all tumors these 3 measurements need to be recorded, using either T1 or T2 weighted images (which ever gives the best estimate of tumor size). The following section describes the methodology.

1. Longest diameter of target lesion(s) should be selected in the axial plane only for CT. For MRI imaging, the longest diameter can be measured from the axial plane or the

plane in which the tumor is best seen or measured, provided the same plane is used in follow up.

2. The longest measurement of the tumor (or width, W) should be determined.
3. The 2 perpendicular measurements should be determined (transverse (T) measurement-perpendicular to the width in the selected plane, and the length (L) – tumor extent in the plane perpendicular to the selected plane)
4. The cystic or necrotic components of a tumor are not considered in tumor measurements. Therefore, only the solid component of cystic/necrotic tumors should be measured. If cysts/necrosis compose the majority of the lesion, the lesion may not be "measurable". Options:
   a. if the cyst/necrosis is eccentric, the W, T and L of the solid portion should be measured, the cyst/necrosis excluded from measurement
   b. if the cyst/necrosis is central but represents a small portion of the tumor (<25%), disregard and measure the whole lesion
   c. if the cyst/necrosis is central but represents a large portion of the tumor, identify a solid aspect of the mass that can be reproducibly measured
5. Leptomeningeal tumor spread is usually not a target lesion, and usually cannot be measured accurately. Presence and location of leptomeningeal tumor spread should be noted, change in extent/thickness assessed on follow up studies.

9.2 Response Criteria for Target Lesions

Comparison of maximal 2-dimentional measurements, TxW (product of the longest diameter [width (W)] and its longest perpendicular diameter [transverse (T)] will be used for Response Criteria for Target Lesions. A maximum of five target lesions will be included in the response criteria.

- Complete Response (CR): The disappearance of all abnormal signal. This includes return to normal size of the brainstem for brainstem lesions.
- Partial Response (PR): ≥50% decrease in size of tumor in comparison to baseline measurements.
- Minor Response (MR): ≥25% decrease in size of tumor in comparison to baseline measurements.
- Stable Disease (SD): No more than 25% increase or decrease in the size of tumor in comparison to baseline.
- Progressive Disease (PD): > 25% increase in the size of the tumor or appearance of new lesions.

9.3 Overall Response

The overall response assessment takes into account response in both target and non-target lesions, and the appearance of new lesions, where applicable, according to the criteria described in the table below (11.2.1). The overall response assessment is shown in the last column, and depends on the assessments of target, nontarget, and new lesions in the preceding columns.

10.0 Criteria for Removal from Study

10.1 Criteria for Removal from Study Therapy

- Patient/parent preference
- Non-compliance with treatment or required observations Physician decision that study is not in patient's best interest
- Uncontrolled allergic reaction to protocol therapy
- Progressive disease
- Completion of all planned therapy (24 cycles)

10.2 Off Study Criteria

- Death
- Loss to follow-up (documented in patient shadow chart if unable to reach patient after three attempts to contact)
- Two-years from start of study treatment +/- 2 months

11.0 Biostatistics

11.1 Estimated Patient Enrollment and Study Duration

11.1.1 Estimated Study Duration

This study will require 32 patients to complete. The treatment period is expected to last from 8 weeks to up to 96 weeks depending on response and tolerability. The University of Michigan sees on average 10 new high-grade glioma patients and 10 refractory/relapsed glioma (grade II-IV) patients annually. Based on our experience, ~1/3 of these patients will harbor relevant DNA alterations to be eligible for this study. Based on these estimates, this study will require approximately 5 years of accrual, with an additional 2 years for completion of study activity and analysis. Any subject who receives two cycles of therapy and undergoes MRI prior to third cycle will be considered evaluable.

11.1.2 Early Stopping Rules

At the end of the first stage, we stop the trial if $Pr(OR>3/data)<0.115$.

Additionally, there will be stopping rules for toxicity to avoid excessive toxicity. If more than one patient develops clinically relevant grade 3 or greater toxicity possibly, probably, or definitely attributed to study treatment, then subsequent patients should start at the first dose reduction level of that agent. If an additional patient on the same treatment regimen develops clinically relevant grade 3 or 4 toxicity possibly, probably, or definitely attributed to study treatment, then the study will be placed on hold for further patient enrollment until further review.

11.2 End Points

11.2.1 Primary End Point:

The primary objective of this study is to test and estimate the efficacy of dasatinib and everolimus. The endpoint in stratum A is progression-free survival (PFS) rate and the endpoint in stratum B is the overall response rate (partial response or better) at two cycles after treatment (**RR2**). Stratum A includes HGG and DIPG patients at diagnosis. Using the standard of care, the estimated median PFS is 12 and 8 months for HGG and DIPG, respectively, based previous clinical trials. Thus, we set 50% as the uninteresting PFS rate for both diagnosis groups. That is, the PFS rate in DIPG is 50% at 8 months (**PFS8**) and the same rate in HGG at 12 months (**PFS12**).[49-51] For both diagnosis groups, we are targeting an improvement of 25% with the combination of dasatinib and everolimus as adjuvant therapy. In Stratum B, the RR at 2 cycles (RR2) in pediatric patients with refractory or relapsed high-grade is glioma is approximately 10% to recently explored therapies,[52,53] and the combination is expected to increase the rate to 25%. It is important to note that the odds ratio (OR) comparing the combination to the standard of care is 3 in both stratums (i.e., the treatment effect is the same). Therefore, OR of 1 is the uninteresting treatment effect, while OR of 3 is the expected treatment effect.

The proposed model is a Bayesian two-stage design. The statistical model in stratum A is $logit(p_A) = \alpha_A + \beta,$ and $logit(p_B) = \alpha_B + \beta$ in stratum B, where the intercepts are log odds of success (RR2, PFS8 or PFS12) in the historical control. That is, $\alpha_A = \log(0.5/(1-0.5), \alpha_B = \log(0.1/(1-0.1)$ and β is log OR (the combination therapy vs standard of care) in both stratum A and B, and p_A and p_B are PFS rate (PFS8 for DIPG or PFS12 for HGG) and RR2 respectively. At the first stage, it's likely that some patients are free of progression and have not reached the observation window (the window is 8 months for DIPG and 12 months for HGG). To incorporate the partial information for these patients into the statistical modelling, we assign a weight, w_i ($0 < w_i < 1$), to each of these patients based upon how long he/she has been followed divided by the observation window. For patients who progressed or have completed the observation window, the weight is fixed to be one. All patients in stratum B have weights equal to one.

In the proposed design, decisions are made based on the posterior distribution of β. At the end of the first stage, we stop the trial if Pr(OR>3/data)<0.115. At the end of the trial, we reject the null hypothesis and declare the new drug is worth of further investigation if Pr(OR>1/data)>0.95. We ran simulation studies to compare the proposed design to Simon's design, with regarding to frequentist operating characteristics, including type I error, power, probability of early stopping (PET) and expected sample size under the null (E(n)). Simon's design cannot incorporate the partial follow-up information, therefore, the interim decision is made until all enrolled patients experience the event or complete the observation window. In the simulation studies, we simulated patients to arrive via a Poisson process such that an average of 5 patients was enrolled per year in each stratum (the arrival rate is the same for DIPG and HGG patients). For RR2, an indicator of success for each patient was drawn from a Bernoulli distribution with the RR2 specified in the

null an alternative hypothesis. For PFS8 and PFS12, survival times were simulated from exponential distributions with rate parameters corresponding to PFS8=50% and PFS12=50% or PFS8=75% and PFS12=75%. The first stage was at the enrollment of $(n1+1)^{th}$ patient. Simulation results based on 5,000 simulated trials were summarized in Table 2. This table shows that the proposed Bayesian design dramatically reduced sample size compared to Simon's MiniMax design,[54] given similar type I error and power. More importantly, the proposed design allows continuous patient enrollment without recruitment pause.

Table 1: Simon's MiniMax Design:[54]

Stratum	**Response rates (H0 vs H1)**	**n1**	**Decision to stop at stage I**	**n**	**Reject H0 at final stage**
A	50% vs 75%	14	# response ≤ 7	23	# response ≥ 16
B	10% vs 25%	22	# response ≤ 2	40	# response ≥ 8

Table 2: Trial operating characteristics

Design	Stratum	Max Sample size	n1	Type I error	Power	PET under H0	E(n) under H0
Simon	**A**	23	14	0.05	0.80	0.60	18
Simon	**B**	40	22	0.05	0.80	0.62	29
Bayesian	**A & B**	32	16	0.05	0.82	0.50	24

For the primary endpoint: We will obtain a posterior distribution for the overall OR using combined data from two stratums as described in the above trial design. Specifically, we will calculate the posterior mean for the OR, along with a 95% credible set. Furthermore, we will summarize the results within each stratum. For stratum A, the median survival and survival probability will be estimated from the Kaplan-Meier method for DIPG and HGG separately. For stratum B, the RR2 will be calculated with a 95% confidence interval.

11.2.2 Exploratory End Points:

11.2.2.1 The toxicity profile will be described, both overall and by grade. Toxicity rates will be tabulated by type and category. Toxicity endpoints will be descriptive and include grading of patient toxicity according to the National Cancer Institute common terminology criteria for adverse events (CTCAE version 4.0). This combination therapy will be considered intolerable if toxicity attributed to study treatment warrants closure as detailed in section 11.1.2 above.

11.2.2.2 Exploratory correlative imaging (high-B value DWI-MRI), genomic studies (circulating tumor DNA), and CSF pharmacokinetics will be reported descriptively.

For DWI endpoint: DWI will be measured at baseline, 2 and 4 cycles. We will correlate the change of DWI from baseline to the change in tumor size measured by MRI. Specifically, a linear mixed effect model with a random effect will be used to evaluate the association while considering the correlation between data measured within the same subject.

For CSF endpoint: CSF and blood will be measured at baseline and 2 cycles. Pearson correlation coefficient will be calculated to assess the association between the change in CSF and blood, and change in tumor size.

12.0 Data Management

Data for patients enrolled on study will be maintained locally in password- protected electronic files or locked file cabinet for the duration of the study, to include reports of patient eligibility criteria and biology testing results, treatment including modifications, all toxicity of therapy, disease evaluations and survival. All files will be maintained by authorized study team members at the University of Michigan.

13.0 Protection of Human Subjects and Data Safety Monitoring Plan

This trial will be monitored in accordance with the NCI approved University of Michigan Comprehensive Cancer Center Data and Safety Monitoring Plan (Appendix A).

The study team will meet quarterly or more frequently depending on the activity of the protocol. The discussion will include matters related to the safety of study participants (SAE/UaP reporting), validity and integrity of the data, enrollment rate relative to expectations, characteristics of participants, retention of participants, adherence to the protocol (potential or real protocol deviations) and data completeness. At these regular meetings, the protocol specific Data and Safety Monitoring Report form will be completed and signed by the Principal Investigator or by one of the co-investigators.

Data and Safety Monitoring Reports will be submitted to the University of Michigan Comprehensive Cancer Center Data and Safety Monitoring Committee on a quarterly basis for independent review.

14.0 Privacy and Confidentiality

Data provided will be treated in strictest confidence. Each subject enrolled will, from that point forward, be identified by a unique identifier (study number). This study number will also be used for any research specimens collected and shipped to analysts outside of the study sites. All records generated will be stored in a locked office area, only accessible to study personnel. Clinical information will be accessed, according to HIPAA requirements, by study personnel to complete study documents, as needed.

15.0 Informed Consent Procedures

Informed Consent will be obtained from appropriate parents or legal guardians of all patients prior

to beginning any study procedures. Assent will also be obtained from patients in adherence with University of Michigan IRB policies.

16.0 Adverse Events (AEs)

16.1 AE Definition:

Adverse Events (AEs) are defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite). This includes all deaths that occur while a subject is on a study. An adverse experience may also be referred to as an adverse event. Abnormalities present at baseline (before starting protocol therapy) are not adverse events, but for this study will be documented. (See 21 CFR 312.32. of the federal regulations covering investigational agents.) This study will track all AEs, regardless of attribution, for each participant.

16.2 Serious AE (SAE) Definition

A Serious Adverse Event (SAE) is any Adverse Event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.
- Events not considered to be serious adverse events are hospitalizations for:
  - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
  - Elective or pre-planned treatment for a pre-existing condition that did not worsen
  - Emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
  - Respite care

16.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

Expected Adverse Events:
Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Unexpected Adverse Events:

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

16.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned 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.

16.5 Reporting

The investigators on this trial will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study. All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms. Subjects will be followed for occurrence of adverse events for 30 days after the last dose of study treatment.

The descriptions and grading scales found in the CTEP Version 4.0 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. The CTEP Version 4.0 of the CTCAE is identified and located on the CTEP website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

Any Unexpected Serious Adverse Events suspected to be possibly, probably or definitely related to study therapy occurring after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment will be reported to the University of Michigan IRB within 7 days of occurrence. Reporting to the University of Michigan IRB will occur on their Adverse Event Reporting Form. If the participant is in long term follow up, report the death at the time of continuing review. Serious adverse events that meet the FDA reporting requirements will be reported to the FDA according to 21CFR312.32(c).

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Appendix A: Data and Safety Monitoring Plan

Oversight Responsibilities
Day-to-day oversight of the trial is provided by the Principal Investigator (PI), Dr. Carl Koschmann. Along with this study's sub-investigators, Dr. Koschmann assures that informed consent is obtained prior to performing any research procedures, that all subjects meet eligibility criteria, and that the study is conducted according to the IRB-approved protocol. The study PI and sub-investigators review all study data and any adverse events (AEs) in real-time, and report all AEs to the IRB according to the approved DSMP.

Data provided will be treated in strictest confidence. Confidentiality throughout the trial is maintained by a unique identifier (study number). Each subject that signs consent, from that point forward, will be identified by their study number. PHI will not be disclosed to the DSMC. Data provided to the DSMC will only be identified by the study number, when necessary.

DSMC Monitoring Procedures
This trial will be monitored in accordance with the NCI approved University of Michigan Comprehensive Cancer Center Data and Safety Monitoring Plan.

The study team will meet quarterly or more frequently depending on the activity of the protocol. The discussion will include matters related to the safety of study participants (SAE/UaP reporting), validity and integrity of the data, enrollment rate relative to expectations, characteristics of participants, retention of participants, adherence to the protocol (potential or real protocol deviations) and data completeness. At these regular meetings, the protocol specific Data and Safety Monitoring Report form will be completed and signed by the Principal Investigator or by one of the co-investigators.

Data and Safety Monitoring Reports will be submitted to the University of Michigan Comprehensive Cancer Center Data and Safety Monitoring Committee on a quarterly basis for independent review.

Appendix B. CYP3A4 Inhibitors

The following list describes medications and food which are strong to moderate inhibitors of CYP3A4.

Strong CYP3A4 Inhibitors	Moderate CYP3A4 Inhibitors	Weak CYP3A4 Inhibitors
≥ 5-fold increase in AUC	≥ 2 but < 5-fold increase in AUC	≥ 1.25 but < 2-fold increase in AUC
atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycins	amprenavir, aprepitant, diltiazem, erythromycin, fluconazole, fosamprenavir, grapefruit juice(a), verapamil	cimetidine