PROTOCOL TITLE: A single-dose study of orally administrated defactinib or avutometinib in patients with glioblastoma

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COORDINATING CENTER: Emory Winship Cancer Institute

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REVISION HISTORY

Revision #	Version Date	Summary of Changes
1	9-22-23	Page 5: Removal of immunology assays and pharmacokinetics from screening period in Defactinib schedule of events Page 6: Removal of immunology assays and pharmacokinetics from screening period in Avutometinib schedule of events.
2	1-31-24	Grammatical updates throughout Section 6.1: Updating blood sample amount and type of tube being used to obtain the sample
3	3-19-24	Grammatical updates throughout Updated eligibility to include active eye disorders
4	4-4-24	Updating accrual numbers to account for screen failures and grammatical updates throughout
5	7-30-24	Updates to schedule of assessments
6	8-30-24	Updated enrollment numbers

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1. Study Summary

Project Title	Identification of treatment concentrations of defactinib and avutometinib for glioblastoma treatment
Project Design	Laboratory investigation of postsurgical glioblastoma specimens after single dose oral administration of defactinib or avutometinib
Primary Objective(s)	• Characterize or describe the ability of an oral dose of defactinib and avutometinib to reach tumor and brain around tumor • Assess the safety of the administration of a single oral dose of defactinib or avutometinib in patients with glioblastoma
Secondary Objective(s)	• Assess the ability of defactinib and avutometinib to inhibition of Pyk2/FAK and MEK/ERK phosphorylation in those samples, respectively • Assess the pharmacodynamics of defactinib and avutometinib
Research Intervention(s)/Interactions	Single dose oral administration
Study Population	Subjects older than 21 years of age diagnosed with glioblastoma (GBM) that require surgery as part of their management
Sample Size	12 patients
Study Duration for individual participants	From time of consent until suture or staple removal, encompassing approximately 3 weeks.
Study Specific Abbreviations/ Definitions	Gliobastoma (GBM)
Funding Source (if any)	Verastem, Inc

Procedures for Subjects receiving Defactinib	**Screening** Day 1-14	**Defactinib** (200mg) Or (400mg)*	**Surgical resection evaluation**	**Post-Surgical monitoring** For at least the 4 hours following surgery	**End of treatment visit** 2 weeks post surgery
Informed consent	X				
Demographics	X				
Medical history	X				
Con. Medication review					
Administer study intervention		X			
Physical exam (including height and weight)	X				X
Electrocardiogram (EKG)[d]	X				
Echocardiogram (ECHO)[e]	X				
Staple removal; physical exam (including skin and oral)					X
Vital signs	X			X	X
Height	X				
Weight	X				
Serum chemistry [a]	X				X[f]
Pregnancy test [b]	X				
Tissue sample extraction			X		
AEs review and evaluation	X ----------				X
Other assessments (e.g., immunology assays, pharmacokinetic)[c]			X		
Complete Case Report Forms (CRFs)	X	X	X	X	X

1.2 Schedule of Assessments

*In Defactinib arm, three subjects will receive 200mg dose. If no dose limiting toxicities occur, three subjects will receive 400mg dose.

a) Metabolic panel, liver function panel including bilirubin level, kidney function panel, QTc evaluation, PT/INR/PTT
b) Serum pregnancy test within 2 weeks of study drug administration (women of childbearing potential)
c) Western blot analysis of ratio of Pyk2, FAK, MEK1/2 and Erk1/2
d) EKG, QTc to occur within 2 weeks of study drug administration
e) ECHO to occur within 2 week of study drug administration
f) Metabolic panel, liver function panel including bilirubin level, kidney function panel, and PT/INR/PTT

*In AVUTOMETINIB arm, three subjects will receive 3.2mg dose. If no dose limiting toxicities occur, three subjects will receive 4mg dose.

Procedures for Subjects receiving AVUTOMETINIB	**Screening**	**AVUTOMETINIB** (3.2mg) Or (4mg)*	**Surgical resection evaluation**	**Post-Surgical monitoring** For at least the 4 hours following surgery	**End of treatment visit** 2 weeks post surgery
Informed consent	X				
Demographics	X				
Medical history	X				
Con. Medication review					
Administer study intervention		X			
Physical exam (including height and weight)	X				X
Electrocardiogram (EKG)	X				
Echocardiogram (ECHO)	X				
Staple removal					X
Vital signs	X			X	X
Height	X				
Weight	X				
Serum chemistry [a]	X				X[f]
Pregnancy test [b]	X				
Tissue sample extraction			X		
AEs review and evaluation	X ----------				X
Other assessments (e.g., immunology assays, pharmacokinetic)[c]			X		
Complete Case Report Forms (CRFs)	X		X	X	X

a) Metabolic panel, liver function panel including bilirubin level, kidney function panel, QTc evaluation, and PT/INR/PTT.
b) Serum pregnancy test within 2 weeks of study drug administration (women of childbearing potential)
c) Western blot analysis of ratio of Pyk2, FAK, MEK1/2 and Erk1/2.

d) EKG, QTc to occur within 2 weeks of study drug administration
e) ECHO to occur within 2 week of study drug administration
f) Metabolic panel, liver function panel including bilirubin level, kidney function panel, and PT/INR/PTT

2. Objectives

This study is designed as a phase 0 clinical trial whose purpose is to estimate the working concentrations of Pyk2/FAK inhibitor defactinib and RAF/MEK clamp avutometinib, as well as the inhibition of Pyk2/FAK and MEK/ERK phosphorylation in tumor tissue and brain around tumor patients with glioblastoma, respectively.

Objectives: (1) estimate (or characterize) the concentration of defactinib or avutometinib that accumulates in the GBM and brain around tumor, (2) assess the safety of the administration of a single oral dose of defactinib or avutometinib in patients with glioblastoma, (3) assess the inhibition of Pyk2/FAK or MEK, Erk signaling in tumor and brain around tumor, respectively, and (4) assess the pharmacodynamics of defactinib or avutometinib in patients with glioblastoma.

Hypothesis: Administration of defactinib or avutometinib inhibits Pyk2/FAK and RAF/MEK signaling in GBMs, respectively.

3. Background

Our previous studies and published literature reports have identified deregulation of FAK/Pyk2 and RAF/MEK signaling in human GBMs, related to overactivation and gain of gene copy number of their up-stream regulators EGFR, PDGFR, and FGFR receptor tyrosine kinases (Nunez et al., 2021; Robinson et al., 2010). Although oncogenic driver KRAS and BRAF mutations are not common in GBMs (Makito et al.,2021), BRAFV600 has been reported in 69% of epithelioid glioblastomas (Andrews et al., 2022). Additionally, a significant 12-fold up-regulation of BRAF and 2-fold up-regulation of RAF-1 were found in the majority of GBM tissue samples compared to normal brain cortex (Lyustikman et al., 2008). NF1 mutations and deletions, which are the hallmark for GBM with mesenchymal molecular signatures (Phillips et al, 2006; Verhaak et al, 2010), have been shown to accelerate Kras -driven tumorigenesis (Behnan et al., 2019; Xiaojing et al., 2019) and are also associated with FAK hyperactivation (Xiaojing et al., 2019). Taking in account the frequency of EGFR mutations (31.54%, according to MyCancerGenome database and Sage Synapse platform: https://www.mycancergenome.org, https://synapse.org/genie), PDGFR mutations (10%) and NF1 mutations (11-19%) in newly diagnosed GBMs together with the observations that two-thirds of primary GBMs eventually develop a transcriptional profile consistent with the mesenchymal GBM molecular-signature upon recurrence (Phillips et al., 2006), RAF and FAK signaling are potential targets in newly diagnosed and recurrent GBMs, and may be especially important in epithelioid glioblastomas.

As it was shown in our previous studies and by others, FAK/Pyk2 and RAF expression in malignant gliomas are essential for the maintenance of tumor proliferation and dispersal

(Lipinski et al., 2008; Loftus et al., 2009; Rolon-Reyes et al., 2015; Paolino et al., 2010; Nunez et

al., 2021). Targeting of FAK/Pyk2, as well as RAF/MEK, in glioma animal models resulted in significant inhibition of tumor growth (Lipinski et al., 2018, Sathornsumetee et al., 2006). However, human clinical trials identified just modest progression-free survival in patients, receiving inhibitors of FAK/Pyk2 or RAF signaling individually (Cloughesy et al., 2006; Liu et al., 2021). Compensatory up-regulation of FAK/Pyk2 signaling was observed in cancer cells in response to RAF inhibition, driving the treatment resistance (Hirata et al., 2015). Co-inhibition of BRAF and FAK/Pyk2 abolished compensatory Erk reactivation, leading to better control of tumor growth.

Avutometinib is a novel RAF/MEK inhibitor (RAF/MEK clamp) that blocks both RAF and MEK via a single molecule and has the advantage of inhibiting downstream Erk signaling more completely than other targeted agents. defactinib is a small molecule focal adhesion kinase (FAK) inhibitor that inhibits parallel pathway signaling and has demonstrated synergy with RAF/MEK inhibitor AVUTOMETINIB (data obtained from preliminary clinical studies, conducted by drug sponsor of the study Verastem Inc). In clinical observations, avutometinib induced elevated FAK phosphorylation (pFAK) as a potential resistance mechanism in the majority of patients, while the combination of avutometinib and defactinib reduced this compensatory pFAK signal, leading to better treatment outcome.

As an overarching concept, **we hypothesize** that with proper treatment development, dual inhibition of RAF/MEK and FAK/Pyk2 signaling with use of combinatorial administration of avutometinib and defactinib for GBM treatment will target and inhibit tumor cell proliferation. As an initial step, pharmacodynamic studies of individual administrations of avutometinib and defactinib in GBM tissues will provide a basis for next step development of combinatorial therapies utilizing Pyk2/FAK and RAF/MEK inhibitors to provide significant treatment benefit.

4. Study Endpoints

Primary Endpoints:

1. Concentration of defactinib that accumulates in the GBM and brain around tumor.
2. Concentration of avutometinib that accumulates in the GBM and brain around tumor.
3. Assessment of safety of defactinib by quantification of the recognized side effects of this agent including fatigue, nausea, diarrhea, vomiting, hyperbilirubinemia, decreased appetite, peripheral edema, dizziness, and headache and by monitoring for new or undescribed adverse events.
4. Assessment of safety of avutometinib by quantification of the recognized side effects of this agent including rash, creatine phosphokinase elevation, visual disturbances,

hypoalbuminemia, and fatigue and by monitoring for new or undescribed adverse events.

Secondary Endpoints:

1. pPyk2 and pFAK phosphorylation in tumor, brain around tumor, and serum.
2. MEK and Erk in tumor, brain around tumor, and serum.
3. Pharmacodynamics of defactinib and avutometinib

Previous clinical trials have identified dose of defactinib with biologic effect as 400 mg twice per day (BID) and maximum tolerated dose as 750 mg given orally (Jones et al., 2015), and the optimum dose of avutometinib as 3.2-4mg twice per week (BIW) (Guo et al., 2020). The same studies identified that maximum observed serum concentration is achieved 1-3 hours after administration for both drugs.

The most frequent treatment-related adverse effects detected for defactinib in previous clinical trials were hyperbilirubinemia, fatigue, decreased appetite, and diarrhea when given for 21 days in two times a day regimen (Jones et al., 2015; Shimizu et al., 2015). Adverse effects detected for avutometinib in previous clinical trials were rash, visual disturbances, hypoalbuminemia, creatinine phosphokinase elevation, and fatigue when given for 21 days in two times a week regimen (Martinez-Garcia et al., 2012; Guo et al., 2020). Our study proposes to use a single dose of defactinib or a single dose of avutometinib one to two hours prior to the tumor resection surgery initiation to allow maximum levels to occur at the time of tumor sampling. As a portion of this study is to assess safety, the study will be started with a lower dose of 200 mg PO single dose for defactinib and 3.2mg PO single dose for avutometinib for a total of three patients each. Then, the dose will be increased to 400 mg PO single dose and 4mg PO single dose (three patients each) if needed, to reach a working concentration of 13.3 nM for defactinib and 190nM for avutometinib in tumor together with the reduction of Pyk2/FAK and MEK/Erk phosphorylation. In the case of severe adverse effects that place the research subject at risk during study participation, either during surgery or during the postsurgical course, the study of higher concentrations will be suspended until the assessment of the cause of the adverse event. If the adverse events are shown to not be related to the defactinib or avutometinib administration, the study will be resumed. If there is reasonable suspicion the adverse events are related to the agents, dosage will either be modified, or the study curtailed.

5. Study Intervention/Investigational Agent

Description: defactinib and avutometinib are non-covalent Pyk2/FAK and RAF/MEK kinase inhibitors with nanomolar potency. Both drugs were in human clinical trials beginning in 2012 and INDs were developed for both drugs.

Dose Escalation Design

Though this is not a phase I study looking for a maximum tolerated or a phase 2 dose, a dose escalation plan is proposed to ensure that subjects are not exposed to excessive toxicity. The initial plan will be for three subjects to receive the lower dose of defactinib (200 mg) and for three to receive the lower dose of AVUTOMETINIB (3.2 mg). If none of the first 3 subjects for each drug experiences dose limiting toxicity, the dose will be increased to the higher planned dose for the next cohort. If one of the first 3 subjects experiences a dose limiting toxicity, the cohort will be expanded to six subjects. If none of the remaining subjects in the expanded cohort experience dose limiting toxicity, the next cohort will be administered the next higher planned dose. If a second subject experiences a dose limiting toxicity, accrual at the dose level should be halted without further escalations. Consultation will then be held with the DSMC, Verastem and the FDA regarding alternative dosing strategies versus study closure. This same 3+3 plan mentioned for the first dose level will apply to the second dose level for defactinib (400 mg) and for AVUTOMETINIB (4 mg).

If no meaningful amount of defactinib or avutometinib is identified in tumor samples at the second dose level, the study will be put on hold and consultations will be held with the DSMC, Verastem and FDA to determine if higher doses, as reported in the investigator's brochure, should be pursued, or if the study should be ended. If no measurable amounts of either defactinib or avutometinib is identified at the two dose levels proposed, and adverse events encountered do not meet criteria for dose limiting toxicity, the investigators will consult with the DSMC, Verastem and the FDA, and propose further dose levels (tentatively 500 mg and 750 mg for defactinib, and 5 mg for avutometinib, based on the maximum explored single doses tests noted in investigator's brochures for each) to the DSMC. Provided there is approval, the study will then continue using the same dose escalation plan, mentioned in the previous paragraph, as was used in lower dose levels. If there is no approval, the study will be stopped. If there is still no measurable drug in tumor after completion of these additional dose levels, the study will be stopped, and consultation held with Verastem and the DSMC regarding further dosing strategies or closing the study.

Drug Administration

The drugs will be given separately, orally in a single dose. Defactinib will be given at lowest dose of 200mg and escalating to 400mg, and avutometinib will be given at 3.2mg and escalating to 4mg 1 to 2 hours before the planned GBM tumor resection surgery. Concentrations and timing are based on data obtained from previous clinical trials.

Drug handling: Avutometinib will be shipped at 2-8C. Defactinib will be shipped at ambient temperature (15-30C) unless it is shipped with avutometinib in which case it can be shipped at 2-8C. Both can be stored at 4C, but for no longer than 24 months after manufacturing, before being given to the subject. The drugs will be used only for consenting subjects and be used only by authorized investigators.

The control of the defactinib and avutometinib will be accomplished by following an established approved Standard Operation Procedure (SOP) at Emory University Investigational Pharmacy (IDS).

IDS will be used according to the pharmacist supplier to control handling, storage, ordering, receiving, and control of drugs and assure compliance with the standards of the U.S. Food and Drug Administration (FDA) and of the study sponsor.

Defactinib and avutometinib are not commercially available, are not controlled substances, and FDA Risk Evaluation and Mitigation Strategy (REMS) status has not been evaluated.
Defactinib and avutometinib are investigational drugs. An Emory Investigator holds the IND.

Procedures and regulations to be followed to comply with sponsor requirements for FDA regulated research include:
21 CFR 11: to ensure that all records are authentic, incorruptible, and confidential, records will be kept in a closed computer system secured with a password. Only authorized personnel from the study group will have access to the password.
21CFR 54: no financial interests of the clinical investigator in the outcome of the study because of the payment or because the investigator has a proprietary interest in the product or because the investigator has an equity interest in the sponsor of the covered study.
21 CFR 210, 211: the drug will be manufactured, processed, and packed in FDA licensed pharmacological facilities and comply with the requirements of the manufacturer of medical products to ensure that there is no risk for subjects due to inadequate safety procedures.
21 CFR 312: defactinib and avutometinib are investigational new drugs for which INDs are in effect and are exempt from the premarketing approval requirements. Labels with the statement "Caution: New Drug - Limited by Federal (or United States) law to investigational use" will be used. The drugs will not be represented in a promotional context or commercially distributed. The use of drugs will not be prolonged after the end of the finding period to support a marketing application. Subjects will not be charged for the use of defactinib or avutometinib.

5.1. Avutometinib Prophylaxis

Prophylactic medications must be used for a minimum of two weeks after receiving the single dose to mitigate against dermatologic toxicities. Hydrocortisone 1% cream, moisturizer, and sunscreen (sun protection factor [SPF] ≥ 50) should be applied topically BID, along with a systemic antibiotic (minocycline 100 mg daily or doxycycline 100 mg BID). Application of topical agents should include the most commonly affected skin areas such as face, scalp, neck, upper chest, and upper back. In addition, patients should be advised to avoid unnecessary exposure to sunlight.
For patients who cannot tolerate a component of the prophylaxis, that component may be eliminated, or another agent could be used.

Patients who develop rash/skin toxicities should be seen by a qualified dermatologist and should receive evaluation for symptomatic/supportive care management. General recommendations for symptomatic care include:

- Pruritic lesions: cool compresses and oral antihistamine therapies
- Fissuring lesions: Monsel's solution, silver nitrate, or zinc oxide cream
- Desquamation: thick emollients and mild soap
- Paronychia: antiseptic bath, local potent corticosteroids in addition to antibiotics; if no improvement, consult dermatologist or surgeon
- Infected lesions: appropriate bacterial/fungal culture-driven systematic or topical antibiotics

6. Procedures Involved

Total 17 subjects will be enrolled in the study. Defactinib will be given to 6 subjects and avutometinib will be given to other 6 subjects orally within 1-2 hours prior to the GBM tumor resection in planned escalating doses of 200mg (three subjects) and 400mg (three subjects) for defactinib, and 3.2mg (three subjects) and 4mg (three subjects) for avutometinib. Patients will be given the medications at the lower doses in alternating format.

6.1 Assessment for Dose Limiting Toxicity

Although this is not a phase I study looking for dose limiting toxicities, observation for these events will be undertaken, and when they occur, used as a guide for dose escalation. Patients will be given the medications at the lower doses in alternating format.

Dose-limiting toxicities will include the following events that are not clearly due to the underlying disease extraneous causes: any death, any non-hematologic toxicity of grade 3 or higher, grade 3 or higher thrombocytopenia with clinically significant bleeding. Excluded will be grade 3 nausea and vomiting, or diarrhea for less than 72 hours with adequate anti-emetic and other supportive care; grade 3 fatigue for more than one week; grade 3 or higher electrolyte abnormality that lasts for up to 72 hours, is not complicated, and resolves spontaneously or responds to conventional medical interventions; and grade 3 or higher amylase or lipase that is not associated with symptoms or clinical manifestations of pancreatitis. If there are reports of adverse effects qualifying as dose limiting toxicities at lower or upper doses, then dosage will either be modified, or the study curtailed.

Subjects will undergo their planned brain surgery in the same way as if they did not participate in the study. No additional treatments or procedures will be performed during the surgery or after.

<u>Tissue sample extraction</u>:
Two samples will be taken:

- Tumor sample: Tissue samples 1.5-2.0 cm3 in approximate size will be separated from the major tumor mass removed from the patient and placed into a tube containing ice-cold phosphate buffered saline solution (PBS) immediately after surgical dissection. The time of the sample resection will be recorded. The samples will be taken from the non-necrotic area of the tumor. This specimen will be placed on dry ice in the OR pathology lab within 30 minutes of harvest and brought to the investigator's laboratory on dry ice at the completion of the surgery.
- Brain around tumor: Tissue samples 0.25-0.5 cm3 approximate size will be taken from normal brain not related to eloquent function around the tumor and placed into a tube containing ice-cold phosphate buffered saline solution (PBS) immediately after surgical dissection. The time of the sample collection will be recorded. This specimen will be placed on dry ice in the OR pathology lab within 30 minutes of harvest and brought to the investigator's laboratory on dry ice at the completion of the surgery.
- Blood Samples: a 10 ml sample in a purple top tube will be obtained during the tumor resection. The time of the blood collection will be recorded. The blood sample will be brought to the investigator's laboratory at the same time as the tissue specimens.

Laboratory procedures:
Once in the laboratory, each tissue sample will be cut into two parts. One part of the tumor sample, normal brain, and the plasma from the blood sample will be sent to Emory Lipidomics core for pharmacodynamic studies for identification of defactinib and avutometinib concentrations in the tissue and serum. The other part of the tissue sample will be used for protein extraction and pharmacodynamic studies. Extracted proteins will be shipped in dry ice to Puerto Rico, Central Caribbean University (UCC) for western blot analysis of ratio of total and phosphorylated forms of Pyk2 and FAK as well as total and phosphorylated MEK1/2 and Erk1/2. Protein samples will be shipped to the Universidad Central del Caribe based on a Material Transfer Agreement.

Participants will be monitored for safety beginning immediately after the drug consumption and during pre- and post-surgical period. In previous clinical trials no reactions that could potentially interfere with tumor resection surgery were reported. However, if such reactions are observed during the pre-surgical period (e.g., fever, nausea, rash, bleeding, pain) the surgery will be delayed until symptoms are resolved.

Post-surgery procedures:
For up to 4 hours (the length of this will be individualized to the subjects recovery needs per standard of care) following surgery the following postsurgical observations will be collected:

- HR, RR, SpO2, BP and Temperature
- Neurological Assessment
- Pain Score
- Assessment of Wound Sites / Dressings
- Presence of drains and patency of same when utilized

- Other complications/assessment findings as patients condition dictates

Frequency of these routine post-surgery observations will be as per standard of care individualized to the given subject.

Upon staple removal and within two weeks post-surgery window, the following monitoring tests must be completed: metabolic panel, liver function panel including bilirubin level, kidney function panel, and when clinically indicated PT/INR/PTT.

Given the risk of dermatitis and stomatitis, a full physical exam including skin and oral exams must be performed at the time of staple removal.

To lessen the probability or magnitude of risks, the study will be performed beginning with the smaller planned dose of defactinib (200 mg) or smallest dose of avutometinib (3.2 mg). If significant adverse reactions will be detected in pre-surgical period, during the surgery, or in post-surgical period through the time of suture or staple removal (usually about 14 days), the study will be paused for the evaluation of adverse reactions. The study will proceed to higher doses only when it is ascertained that the lower dose does not cause significant health complications or risks for subjects.

No additional drugs and devices outside of the standard treatment practice, except for defactinib or avutometinib, will be used in the management of the participants.

Subject data will be analyzed and reported in an anonymous manner. Only information related to age, sex, race, associated diagnosis, and molecular markers will be collected.

Drug specific investigational data collected during the study: concentration of defactinib or avutometinib in the serum, resected GBM tissue samples, and brain around tumor samples will be measured after consumption of indicated doses of defactinib or avutometanib respectively. This will identify the normal tissue and tumor uptake of defactinib and avutometinib. Western blot analysis of total and phosphorylated versions of the Pyk2, FAK, MEK1/2 and Erk1/2 in the normal tissue and tumor specimen will be correlated with the dosage and serum concentration of defactinib and avutometanib administered and in this manner will identify the amount of defactinib and avutometanib needed for meaningful biologic effect.

No long-term follow-up is proposed for the study.

7. Statistical Analysis Plan

This is a two-arm study to assess the ability of defactinib and avutometanib to reach Glioblastomas (GBM) and the brain tissue around them. Defactinib will be given to 6 subjects, and avutometanib will be given to 6 subjects orally 1-2 hours prior to the GBM tumor resection in planned escalating doses of 200mg (three subjects) and 400mg (three subjects) for defactinib, and 3.2mg (three subjects) and 4mg (three subjects) for avutometanib. Patients will be given the medications in an alternating format. A monitoring period of 21 days will be allowed to elapse after the last subject receives the lower dose to allow for observation for dose limiting toxicities.

Summary statistics of drug concentrations in tumor, brain around tumor, and in concurrent serum sample, as well as Pyk2/FAK, MEK/Erk phosphorylation in tumor and brain around tumor will be reported using descriptive statistics including mean, median, interquartile range, minimum/maximum, and standard deviation. Patient demographics will be summarized descriptively. 95% confidence intervals will be reported, unless otherwise specified. All tests will be two-sided, and a significance level of 0.05 will be considered.

7.1 Sample size considerations:

Number of Participants Enrolled: In this initial study of these agents in humans with glioblastoma, we will be recruiting up to 17 participants over a period of approximately 12 months. Defactinib will be given to 6 subjects and avutometanib will be given to other 6 subjects orally 1-2 hours prior to the GBM tumor resection in planned escalating doses of 200mg (three subjects) and 400mg (three subjects) for defactinib, and 3.2mg (three subjects) and 4mg (three subjects) for avutometanib. We have found a sample size of 3 (per dose and per drug) to be sufficient for characterizing concentration of study drugs in prior Phase 0 studies [Quillin et al., 2020]. The number of subjects available for recruitment is based on the principal investigator's estimate from his neurosurgical oncology practice. This number (17) is an estimate of the number of cases with (1) imaging consistent qualifying histology (or previous diagnosis of glioblastoma) that (2) require craniotomies for surgical resection as the next step in their therapy that can be accrued over approximately a year at our institution. Based on our accrual experience, it is anticipated that some subjects will not have final pathology showing glioblastoma or will fail screening. Thus, it is expected that to obtain 12 individuals meeting screening criteria, actually having a glioblastoma and whose tissue analysis is meaningful, as many as 17 subjects may need to be enrolled.

7.2 Primary endpoints:

Measurement of defactinib and avutometanib Levels in Tumor, Brain Around Tumor, and Serum: Defactinib concentration will be measured in a sample of the glioblastoma, brain around the glioblastoma, and in a serum sample from each subject receiving

defactinib. Avutometanib concentration will be measured in a sample of the glioblastoma, brain around the glioblastoma, and in a serum sample from each subject receiving avutometanib. Descriptive statistics, such as mean and standard deviation, will be generated with these results. Concentration will be compared between dose levels within study drug using two-sample t-tests or non-parametric equivalents such as Mann Whitney U tests.

Safety: As this is the first study of these agents in subjects with brain tumors, the nature and incidence of adverse events and risk is not known in a quantitative manner. In individuals with other forms of cancer, regular use over time has been associated with adverse events such as fatigue, nausea, diarrhea, vomiting, hyperbilirubinemia, decreased appetite, peripheral edema, dizziness, and headache for defactinib [Gerber et al., 2019] and rash, creatine phosphokinase elevation, hypoalbuminemia, and fatigue for avutometanib [Guo, 2020]. As this study will only be using one dose of the drug, the likelihood of these adverse events is smaller than reported in these other disease types. The incidence of these events is estimated to be in the range of 0.5% or less for these events combined. Safety stopping rules are described below. Summary statistics will include frequencies and percentages of these adverse events.

7.3 Secondary endpoints

Pyk2 and FAK phosphorylation: To explore the effect on its proposed targets, samples from patients receiving defactinib will be processed to measure pPyk2 and pFAK phosphorylation. These results will be summarized with descriptive statistics. These results will be compared to current and historical control samples of glioblastoma (collected in our previous human GBM specimens' studies) and analyzed in parallel with study subject samples and run on one western blot membrane to reduce technique variation (Nuñez RE, et al., 2021). As mentioned elsewhere in this protocol we anticipate there will be 12 sets of subject specimens. The mean phosphorylation will be compared between groups using two-sample t-test. s or non-parametric equivalents such as Mann Whitney U tests. Dose levels will be compared to a historical control, both alone and combined.

MEK1/2 and ERK phosphorylation: To explore the effect on its proposed targets, samples from patients receiving avutometanib will be processed to measure MEK1/2 and Erk1/2 phosphorylation. These results will be summarized with descriptive statistics. The results will be compared to historical glioblastoma specimens as described above for defactinib. Phosphorylation will be compared between dose levels within study drug using two-sample t-tests or non-parametric equivalents such as Mann Whitney U tests.

Pharmacodynamics: Provided samples of the tumor, brain around tumor, and serum provide technically valid data, the drug concentration and pharmacodynamic data normality diagnostics will be done with Shapiro-Wilk estimations to evaluate the raw statistical distribution of our datasets. Frequency and SEM graphs will be calculated for univariate purposes. Two-sample t-tests or Mann-Whitney U tests will be used to compare the mean (or median) differences between dosage groups for each drug.

7.4 Analysis populations

Pharmacodynamics/safety: All patients undergoing administration of defactinib or avutometinibprior to surgical resection of their glioblastoma.

7.5 Safety stopping rules

To allow for the ability to take steps to assess and minimize adverse events, the investigators will stop accrual if one patient experiences at least one severe adverse event (as defined by standard CTCAE 5.0 criteria) occurrence possibly or probably related to the drug over a series of 3 consecutive subjects for a given agent for a given dose. Adverse events and severe adverse events attributable to the tumor, the surgery for it, or the standard of care drugs used before and after surgery will not be included in this consideration. The severe adverse events will then be studied by the investigators and steps to mitigate them for future cases will be outlined for the WCI Data Safety Monitoring Committee (DSMC) and Verastem. Once these steps are approved by the WCI Data Safety Monitoring Committee and Verastem accrual will be resumed. If a remedy cannot be determined to mitigate the toxicity, accrual will stop. The initial plan is for only 3 patients maximum will be treated at a given dose level for a study drug. However, per the Dose Escalation Design, expansion to 6 patients in one dose group may occur if severe toxicity that is successfully treated and whose further occurrence can be mitigated per an agreed upon plan with the DSMC and Verastem. In the case that a lower dose level provides satisfactory pharmacokinetic and pharmacodynamic data before reaching 6 patients, a plan to move to a higher dose level after fewer than 6 patients the study will be put on hold and a plan will be presented to the DSMC and Verastem to expedite study completion.

The study safety window will be from the time of drug administration just prior to surgery until suture or staple removal, which is the stated off study time point.

In order to look for the commonly encountered toxicities listed below, the subjects will undergo the following laboratory studies at the time of suture or staple removal: metabolic panel, lever

function panel including bilirubin level, kidney function panel, and when clinically indicated PT/INR/PTT. In addition, at the time of suture or staple removal, the subject will undergo full physical exam including skin and oral exam, with emphasis on looking for dermatitis and stomatitis.

Commonly encountered toxicities for defactinib that will be monitored for serious adverse events (Grade 3 toxicity or above by CTCAE 5.0 criteria) will be fatigue, nausea, diarrhea, vomiting, hyperbilirubinemia, decreased appetite, peripheral edema, dizziness, and headache (not attributable to the brain tumor or it's treatment). Though the these are the most likely toxicities based on the investigator's brochure, other toxicities will also be recorded and addressed with therapy as appropriate for the event. As mentioned above, if a remedy cannot be determined to mitigate the toxicity, accrual will stop.

Less commonly encountered toxicities for defactinib that will be monitored for that are not clearly due to the underlying disease or extraneous causes: any death; any non-hematologic toxicity of grade 3 or higher; grade 3 or higher thrombocytopenia with clinically significant bleeding; and neutropenic fever.

Though the these are the most likely toxicities based on the investigator's brochure, other toxicities will also be recorded and addressed with therapy as appropriate for the event. As mentioned above, if a remedy cannot be determined to mitigate the toxicity, accrual will stop.

8. Data Specimen Banking

No data or specimens will be banked for future use.

9. Sharing of Results with Participants

Individual subject results will not affect the standard treatment methods for the patient. For this reason, the individual results will be shared with the subject upon request only.
Information gained during the research project may be published in the form of reports or journal articles. However, the personal information will not be identified in any way in those publications.
No incidental findings are anticipated.

10. Study Timelines

• The duration of an individual participant's participation in the study is within a standard timeline of pre- and post-surgical observation, with them coming off study at the time of suture/staple removal.

• The duration anticipated for enrolling all study participants is one year.

• The estimated date for the investigators to complete this study and primary analyses is 18 months from the beginning of the study

11. Inclusion and Exclusion Criteria

Inclusion criteria:

1. New or recurrent glioblastoma diagnosed by neuroimaging techniques for which surgical resection is indicated.
2. Age older than 21 years
3. An Eastern Cooperative Group (ECOG) performance status ≤ 1.
4. Must have adequate organ function defined by the following laboratory parameters:
   a. Adequate hematologic function including hemoglobin (Hb) ≥ 9.0 g/dL; platelets ≥100,000/mm^3; and absolute neutrophil count (ANC) ≥ 1500/mm^3). If a red blood cell transfusion has been administered the Hb must remain stable and ≥ 9.0 g/dL for at least 1 week prior to first dose of study therapy.
   b. Adequate hepatic function: (i) total bilirubin ≤ 1.5 × upper limit of normal (ULN) per the institution; patients with Gilbert syndrome may enroll if total bilirubin < 3.0 mg/dL (51 µmole/L); (ii) alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 × ULN (or < 5x ULN in patients with liver metastases).
   c. Adequate renal function with a creatinine clearance rate of ≥ 50 mL/min as calculated by the Cockcroft-Gault formula or serum creatinine of ≤ 1.5 × ULN.
   d. International normalized ratio (INR) ≤ 1.5 and partial thromboplastin time (PTT) ≤ 1.5 x ULN in the absence of anticoagulation or therapeutic levels in the presence of anticoagulation.
   e. Albumin ≥ 3.0 g/dL (451 µmole/L).
   f. Creatine phosphokinase (CPK) ≤ 2.5 x ULN.
   g. Adequate cardiac function with left ventricular ejection fraction ≥ 50% by echocardiography (ECHO) or multiple-gated acquisition (MUGA) scan.
5. Baseline QTc interval < 460 ms for women and ≤450 ms for men (average of triplicate readings) (CTCAE Grade 1) using Fredericia's QT correction formula. NOTE: This criterion does not apply to subjects with a right or left bundle branch block.
6. Adequate recovery from toxicities related to prior treatments to at least Grade 1 by CTCAE v 5.0. Exceptions include alopecia and peripheral neuropathy grade ≤ 2.
7. Male and female patients with reproductive potential agree to use highly effective method of contraceptive (per Clinical Trial Facilitation Group [CFTG] recommendations) during the trial and for 3 months following the last dose of 6766 for male patients, and 1 month following the last dose of 6766 for female patients.

Adults unable to consent will be included in the study with the condition of their Legal representative providing informed consent.

Exclusion criteria:

1. Clinically significant active gastrointestinal abnormalities, requirement for systemic anticoagulation or potent CYP 2C8 inhibitors, and history of clinically significant cardiac or pulmonary disorders
2. Minors will be excluded from the investigation. Glioblastoma is the major form of brain cancer in people over 50 years old. Pediatric cases of glioblastoma are relatively rare. Besides this, there are crucial molecular differences between adult and pediatric gliomas. Our preliminary data for proposed investigation were obtained on GBM specimens and cultures developed from GBM tissues donated by adult subjects. Results of investigation of adult glioma tissue cannot simply be extrapolated to children. Therefore, our primary research focus is the investigation of GBM in adults. If appropriate, a separate, age-specific study in children will be performed.
3. Pregnant women will be excluded from the study as altered hormonal and immunological status can affect the study results.
4. Prisoners will be excluded from the study.
5. Systemic anti-cancer therapy within 4 weeks of the first dose of study therapy.
6. History of prior malignancy, with the exception of curatively treated malignancies or malignancies with very low potential for recurrence or progression.
7. Major surgery within 4 weeks (excluding placement of vascular access), minor surgery within 2 weeks, or palliative radiotherapy within 1 week of the first dose of the investigational agent (defactinib or avutometinib).
8. Exposure to medications (with or without prescription), supplements, herbal remedies, or foods with potential for drug-drug interactions with the investigational agent (Defactinib or Avutometinib) within 14 days prior to the first dose of the investigational agent (Defactinib or Avutometinib) and during the course of therapy, including:
    a. **Avutometinib**: strong CYP3A4, inhibitors or inducers, due to potential drug-drug interactions with Avutometinib. For additional guidance see https://www.fda.gov/drugs/drug-interactions-labeling/drug- development-and-drug-interactions-table-substrates-inhibitors-and-inducers
    b. **Defactinib:** strong CYP3A4, CYP2C9, and P-glycoprotein (P-gp) inhibitors or inducers, due to potential drug-drug interactions with Defactinib. For additional guidance see https://www.fda.gov/drugs/drug- interactions-labeling/drug-development-and-drug-interactions-table-substrates- inhibitors-and-inducers
9. Known hepatitis B, hepatitis C or human immunodeficiency virus (HIV) infection that is active and/or requires therapy.
10. Active skin disorder that has required systemic therapy within the past 1 year.
11. History of rhabdomyolysis.
12. Concurrent ocular disorders:

a. Patients with actively treated glaucoma, retinal vein occlusion (RVO), including predisposing factors for RVO, including uncontrolled hypertension, and uncontrolled diabetes.
b. Patients with current evidence of visible retinal pathology that is considered a risk factor for RVO, intraocular pressure > 21 mm Hg as measured by tonometry, or other significant ocular pathology and anatomical abnormalities that increase the risk for RVO.
c. Patients with active corneal erosion (instability of corneal epithelium), corneal degeneration, active or recurrent keratitis, and other forms of serious ocular surface inflammatory conditions.

13. Concurrent congestive heart failure, prior history of class III/ IV cardiac disease (New York Heart Association [NYHA]), myocardial infarction within the last 6 months, unstable arrhythmias, unstable angina, or severe obstructive pulmonary disease.
14. Patients with the inability to swallow oral medications or impaired gastrointestinal absorption due to gastrectomy or active inflammatory bowel disease.
15. Patients with a history of hypersensitivity to any of the inactive ingredients (hydroxypropylmethylcellulose, mannitol, magnesium stearate) of the investigational product.

12. Vulnerable Populations

This research does not involve minors, pregnant women, baseline cognitively impaired adults, or prisoners.

13. Local Number of Participants

A total of 17 participants will be enrolled (consented) to reach our recruitment goal of 12 patients study wide: accrual will continue until there are 3 participants completing the study in each experimental group of defactinib 200mg, defactinib 400mg, avutometanib 3.2mg and avutometanib 4mg. This number of participants is needed to provide meaningful descriptive data for the research procedures. Sex and race will not be taken in consideration during the screening and enrollment. Due to this we anticipate that percentage of the participants according to sex and race will reflect demography in the area of enrollment.

14. Recruitment Methods

Patients will be informed about the study by the investigators and/or a designated certified Research Coordinator at Emory Clinic, the Winship Cancer Institute, or Emory Hospital by personal communication and by the provided Informed Consent Form. Patients scheduled for GBM tumor resection surgery at Emory University Hospital will be approached. Eligibility will be

determined by the investigators and Research Coordinator based on the specified inclusion/exclusion criteria.

Laboratory researchers will not have any personal information about the patients during recruitment and study.

Patients will have the possibility to contact the Principal Investigator to clarify all possible questions before and during the investigation.

15. Withdrawal of Participants

Participants can be withdrawn from the study if the brain tumor surgery is canceled or if surgery is postponed for longer than 6-7 hours after DEFACTINIB or AVUTOMETINIB administration. In these cases, the blood samples and samples of the resected tumor and brain will not be taken for analysis.

16. Risk to Participants

Previous clinical trials have identified the optimum dose of defactinib as 400 mg (Jones et al., 2015) and the optimum dose for avutometanib as 3.2mg (Guo et al., 2020) for non-brain tumor malignancies. The most frequent treatment-related adverse effects detected in previous clinical trials were hyperbilirubinemia, fatigue, decreased appetite, and diarrhea for defactinib when given for 21 days (two times a day) (Jones et al., 2015; Shimizu et al., 2015) and rash, creatinine phosphokinase elevation, hypoalbuminemia, and fatigue for avutometinibwhen given for 21 days 2 times a week (Guo et al., 2020). However, these reactions were mostly not observed at lower concentrations, such as 200mg and 3.2 respectively for each drug. Our study proposes a single dose of defactinib or avutometinib. For safety, the study will be started with the lowest dose of 200 mg, and the dose will be increased till 400 mg if the identified concentration of defactinib in the tumor together with the reduction of Pyk2 and FAK phosphorylation is not achieved at lower doses. The same dose escalation from 3.2 to 4 mg is proposed for Avutometanib is proposed if identified drug concentration in the tumor together with the reduction of MEK1/2 and Erk1/2 phosphorylation is not achieved at 3.2mg dose. To minimize risk to subjects and considering adverse reactions observed in previous clinical trials, subjects with clinically significant gastrointestinal abnormalities, anticoagulation disorders, and history of clinically significant cardiac or pulmonary disorders will be excluded from the study. In case of severe adverse effects, that place subjects at risk related to defactinib or avutometinib administration, appear during surgery or post-surgical rehabilitation, the study with given and higher concentrations will be suspended until the cause of defactinib or avutometinib complication is understood and

addressed. We expect the low risk of injury or severe side effects from all study procedures. The patients will be having surgery regardless of their participation in this study.

Laboratory research and data reporting will involve the use of coded private information and biological specimens only. Rights and welfare of subjects are expected to be at minimal risk.

17. Potential Benefits to Participants

Participants will not receive any financial or medical benefits for their participation.

18. Compensation to Participants

No monetary compensation will be provided to participants

19. Data Management and Confidentiality

The clinical researchers will have access to personal information about the patients. The designated certified Research Coordinator at Emory Hospital will provide the laboratory researchers with the unique Identification Number of each sample. All documents containing the information about the names of the patients and their Identification Number will be kept at the Clinical Trials Office at the Winship Cancer Institute in a password protected computer in a safely locked office.

All members of the research group, who will work with biospecimens, have certification in Biomedical Research with Humans subjects and Health Information Privacy and Security (HIPS) for Clinical Investigators training. MDs, that are professionally involved in both, patient's pre- and post-surgical monitoring, surgery procedure, and by these have access to patient's identifiable information, and who are also involved in the study, will maintain patient confidentiality. In this certificate they will indicate that they never pass or make it available by any way the identifiable personal information related to biospecimens to the research group.

Specimens will be provided to the research group together with the Collection Instruments on basic patient data, where gender, age, ethnicity, diagnosis, and time of defactinib introduction, and tumor resection surgery will be indicated.

Biospecimens will be processed immediately for the research procedures and are not intended to be stored. The access to biospecimens will have a Clinical Research Coordinator (who will pass specimens to the research team) and the member of the research group, who will process specimens for protein extraction and send them to Emory Lipidomics Core. This member of the

research group is to be named later and will be required to have Human Subjects Research and HIPAA training and certifications. Extracted proteins for PYK2, FAK, MEK and Erk analysis, which will only have tissue identification number, will be shipped frozen to Puerto Rico, Central Caribbean University (UCC) overnight UPS. The tissue intended for pharmacokinetic and pharmacodynamic analysis will be sent to Emory Lipidomics core for Mass-Spectrometry

If a participant declines to participate in defactinib or avutometanib treatment and following surgery tissue collection, the participant will not be assigned a study ID number and the study coordinators/data collectors will refrain from collecting any data on the participant. If the participant agrees to participate in defactinib or avutometanib treatment and following the surgery tissue collection, but refused to provide any related information, as age, gender, ethnicity, the participant will be assigned a study ID number and the study coordinators/data collectors will be instructed to collect data only on those aspects of the study to which the participant has agreed to participate. These procedures will help prevent unauthorized inclusion of the patient's data in the database.

Scientific data obtained in the proposed investigation will be subject to publication in medical and scientific journals. The personal data regarding the patients providing the specimens will not be published.

20. Provisions to Monitor the Data to Ensure the Safety of Participants

☒**More than minimal risk** – Continue below.

Mark the risk categorization, as determined by the Data and Safety Monitoring Questionnaire, which applies to your study below:

Select one of the following (do not delete this table; review the guidance document for definitions):	
☐ Medium Complexity	
☒ High Complexity Category A	
☐ High Complexity Category B *If choosing this category for a study under an IND or IDE because you believe the study intervention does not significantly impact morbidity or mortality, please provide your rationale:*	

DSMP Requirement	How this Requirement is Met	Frequency	Responsible Party(ies)
Real-time review of participant data during initial data collection.	This requirement will be met per Winship's NCI approved DSMP	This will occur every time new information is obtained.	SI/study team
Site Monitoring at pre-determined intervals: The Principal Investigator has a responsibility to ensure that the study is following all aspects of the protocol.	This requirement will be met per Winship's NCI approved DSMP	biannually	DSMC
100% review of regulatory files	DSMC monitors will review the protocol, amendments, informed consent documents, IRB submissions and meet with the principal investigator for clarification of study objectives	Reviewed at first and close-out visits	DSMC
100% review of consent forms	Monthly QA check of 5-10 randomly selected consents to validate Central Subject Registration (CSR) and PRMS to conduct QA consent checks in real time as subjects are registered in OnCore vis CSR process	biannually	PRMS, QM
Review of credentials, training records, the delegation of responsibility logs (if applicable)	Clinical trials monitor will review the electronic regulatory binder and compare them against the staff listed on the DOA log. The monitor also reviews the site's source documents to ensure that all study staff have been properly listed on the DOA and have corresponding documentation (CV, ML, GCP certs, training log) filed in the electronic regulatory study binder	biannually	DSMC
Comparison of case report forms (CRF) to source documentation	The PI is responsible for ensuring that instances of egregious data insufficiencies	biannually	DSMC

for accuracy and completion	that may impact the scientific integrity of the trial		
Review of documentation of all adverse events	During the monitoring process, the DSMB reviews trial safety data for stopping rules, deviations, study amendments, accrual rates and monitoring reports for therapeutic investigator-initiated clinical trials and any other trial as deemed necessary	biannually	DSMC
Monitoring of critical data points (eligibility, study endpoints, etc.)	The assigned monitor will randomly select subject(s) for review based on parameters in Table 1 or 2 as noted above. Although the principal investigator and applicable study team members will receive notification of trial monitoring in advance, the subject selection will not be revealed in advance of the monitoring visit.	biannually	DSMC
Laboratory review of processing and storage of specimens	The assigned monitor will randomly select subject(s) for review based on parameters in Table 1 or 2 as noted above	Reviewed at first and close-out visits and at least biannually	DSMC
Assessment of laboratory specimens stored locally	If accrual at time of initial monitoring is> IO but :S 20, I 0% of subjects will be monitored at minimum. Thereafter, monitoring will not occur unless accrual reaches 30	Reviewed at first and close-out visits and at least biannually	DSMC
Test article accountability review	In addition to a comprehensive review of available toxicity data, the DSMC reviews all internal monitoring reports of trials under its purview	Reviewed at first and close-out visits and at least biannually	DSMC
Accountability logs, dispensing records, and other participant records	If accrual at time of initial monitoring is> IO but :S 20, I 0% of subjects will be monitored at minimum. Thereafter,	At least biannually	DSMC

	monitoring will not occur unless accrual reaches 30.		
For FDA regulated studies, the following requirements apply:	monitoring activities meet the FDA's requirements as delineated in 21 CFR 50, 21 CFR 56, 21 CFR 812 for studies conducted under an IDE and 21 CFR 312 for studies conducted under an IND.	biannually	DSMC
Monitoring methods (may include centralized, on-site, and self-monitoring)	The internal monitoring team is independent from any study protocol and does not perform any trial-related specific duties in order to uphold an unbiased approach to study monitoring. Oversight of the monitoring process and identification/assignment of studies for monitoring is provided by the Manager of the Internal Monitors.	biannually	DSMC
*For international studies, you are required to engage a CRO that is working in the site country and/or to consult with Emory's legal counsel regarding compliance with the country's clinical research regulations.			

The Data and Safety Monitoring Committee (DSMC) of the Winship Cancer Institute will provide oversight for the conduct of this study. The DSMC functions independently within Winship Cancer Institute to conduct internal monitoring functions to ensure that research being conducted by Winship Cancer Institute Investigators produces high-quality scientific data in a manner consistent with good clinical practice (GCP) and appropriate regulations that govern clinical research. Depending on the risk level of the protocol, the DSMC review may occur every 6 months or annually. For studies deemed High Risk, initial study monitoring will occur within 6 months from the date of the first subject accrued, with 2 of the first 5 subjects being reviewed. For studies deemed Moderate Risk, initial study monitoring will occur within 1 year from the date of the first subject accrued, with 2 of the first 5 subjects being reviewed. Subsequent monitoring will occur in routine intervals per the Winship Data and Safety Monitoring Plan (DSMP).

The DSMC will review pertinent aspects of the study to assess subject safety, compliance with the protocol, data collection, and risk-benefit ratio. Specifically, the Winship Cancer Institute Internal Monitors assigned to the DSMC may verify informed consent, eligibility, data entry, accuracy and availability of source documents, AEs/SAEs, and essential regulatory documents. Following the monitoring review, monitors will provide a preliminary report of monitoring findings to the PI and other pertinent individuals involved in the conduct of the study. The PI is

required to address and respond to all the deficiencies noted in the preliminary report. Prior to the completion of the final summary report, monitors will discuss the preliminary report responses with the PI and other team members (when appropriate). A final monitoring summary report will then be prepared by the monitor. Final DSMC review will include the final monitoring summary report with corresponding PI response, submitted CAPA (when applicable), PI Summary statement, and available aggregate toxicity and safety data.

The DSMC will render a recommendation and rating based on the overall trial conduct. The PI is responsible for ensuring that instances of egregious data insufficiencies are reported to the IRB. Continuing Review submissions will include the DSMC recommendation letter. Should any revisions be made to the protocol-specific monitoring plan after initial DSMC approval, the PI will be responsible for notifying the DSMC of such changes. The Committee reserves the right to conduct additional audits if necessary.

The research involves more than Minimal Risk to participants, as oral administration of a defactinib or avutometinib single dose is proposed. The Winship Cancer Institute Data and Safety Monitoring Committee will monitor this Phase 0 clinical trial. The DSMC functions independently within Winship Cancer Institute to conduct internal monitoring functions to ensure that research being conducted by Winship Cancer Institute Investigators produces high- quality scientific data in a manner consistent with good clinical practice (GCP) and appropriate regulations that govern clinical research. Initial study monitoring will occur within 6 months from the date of the first subject accrued, with 2 of the first 5 subjects being reviewed. The DSMC will review pertinent aspects of the study to assess subject safety, compliance with the protocol, data collection, and risk-benefit ratio. Specifically, the Winship Cancer Institute Internal Monitors assigned to the DSMC may verify informed consent, eligibility, data entry, accuracy and availability of source documents, AEs/SAEs, and essential regulatory documents. Following the monitoring review, monitors will provide a preliminary report of monitoring findings to the PI and other pertinent individuals involved in the conduct of the study. The PI is required to address and respond to all the deficiencies noted in the preliminary report. Prior to the completion of the final summary report, monitors will discuss the preliminary report responses with the PI and other team members (when appropriate). A final monitoring summary report will then be prepared by the monitor. Final DSMC review will include the final monitoring summary report with corresponding PI response, submitted CAPA (when applicable), PI Summary statement, and available aggregate toxicity and safety data. The DSMC will render a recommendation and rating based on the overall trial conduct. The PI is responsible for ensuring that instances of egregious data insufficiencies are reported to the IRB. Continuing Review submissions will include the DSMC recommendation letter. Should any revisions be made to the protocol-specific monitoring plan after initial DSMC approval, the PI will be responsible for notifying the DSMC of such changes. The Committee reserves the right to conduct additional audits if necessary.

Data and Safety Monitoring plan:

- Subjects will be monitored hourly after oral administration of defactinib or avutometanib for stomach pain, nausea, dizziness, fatigue, diarrhea as these effects were reported in previous clinical trial studies with use of defactinib and avutometanib (Jones et al., 2015; Guo et al., 2020). If additional symptoms appear, they will be recorded and addressed by PI and Clinical Research coordination staff.
- During the surgery, possible related adverse effects (such as excess bleeding, heart, and pulmonary complications) will be recorded and reported by the PI or the operating surgeon.
- In the post-surgical period subjects will be monitored by the hospital personnel and Clinical Research coordinator according to routine postsurgical care protocol. All possible related adverse effects will be reported to PI.
- A subject will be removed from study participation in case of adverse reactions leading to delay of surgery for longer than 6-7 hours. The decision will be made by the PI.
- If there are adverse reactions, as evaluated by the primary physician are more than mild (grade 2) and are suspected to be related to defactinib or avutometinib administration, the PI will postpone further accrual to the study for the evaluation of the risk for future subjects. If risk is found to be significant, the administered dose will be reduced in consultation with the Data Safety Monitoring Committee for other participants and study will be resumed. If risk is evaluated as not significant, the study will be continued as planned. If a severe adverse effect is repeated in another subject even after dose modification, the PI will stop the study for further evaluation of mechanisms of the severe adverse reaction restarting or curtailing the study will be carried out in consultation with the Data Safety Monitoring Committee.
  If no measurable amounts of either defactinib or avutometanib is identified at the two dose levels proposed, and adverse events encountered have been grade 3 or less, the investigators will consult with Verastem and propose further dose levels (tentatively 500 mg and 750 mg for defactinib, and 5 mg for avutometinib, based on the maximum explored single doses tests noted in investigator's brochures for each) to the DSMC. Provided there is approval, the study will then continue using the same scheme as was used in lower dose levels. If there is still no measurable drug in tumor after completion of these additional dose levels, the study will be stopped, and consultation held with Verastem and the DSMC regarding further escalation strategies or closing the study.

Reporting Requirements for IND holder

For Investigator-sponsored IND studies, reporting requirements for the FDA apply in accordance with the guidance set forth in 21 CFR, Part 312.32. Events meeting the following criteria need to be submitted to the FDA as Expedited IND Safety Reports.

7 Calendar-Day Telephone or Fax Report

The Sponsor-Investigator is required to notify the FDA of a fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of *investigational agents*. An unexpected adverse event is one that is not already described in the

most recent Guidance for Investigator section of the Investigator's Brochure. Such reports are to be telephoned or faxed to the FDA, within 7 calendar days of the first learning of the event.

15 Calendar-Day Written Report

The Sponsor-Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious unexpected adverse event that is considered reasonably or possibly related to the use of investigational agent.

Written IND Safety Reports with analysis of similar events are to be submitted to the FDA, within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500 Form but alternative formats (e.g., summary letter) are acceptable.
FDA Fax number of IND Safety Reports: 1-(800)-FDA-1078.
The IND sponsor will also assess whether the event constitutes an unanticipated problem posing risks to subjects or others (UP). This assessment will be provided to the Emory University IRB, which, in turn will make a final determination. If the Emory IRB determines an event is a UP it will notify the appropriate regulatory agencies and institutional officials.

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

21. Provisions to Protect the Privacy Interest of Participants

The Clinical Research Coordinator will have access to subjects and their personal information. The Clinical Research Coordinator will provide subject information related to the study, answer questions, and clarify procedures to be performed. If a subject wants to contact the PI directly for deeper clarifications, they can do it by the patient portal, phone or in a personal meeting.

The laboratory researchers will not have access to any sources of personal identifying information about the participants. The Research Coordinator will provide researchers with the unique Identification Number of each sample but not with the name or personal identifying information of the patient. All documents containing the information about the names of the patients and their Identification Number will be kept at the Clinical Trials Office at the Winship Cancer Institute in a password protected computer.

22. Economic Burden to Participants

Participants will not receive any payments in relation to their participation in the study.

23. Informed Consent

The initial informed consent discussion will occur in Winship Cancer Institute or the Emory Clinic.

At Winship Cancer Institute, the informed consent is an ongoing, interactive process rather than a one-time information session. The consent form document is designed to begin the informed consent process, which provides the patient with ongoing explanations that will help them make educational decisions about whether to begin or continue participating in the trial. The research team knows that a written document alone may not ensure that the patient fully understands what participation means. Therefore, the research team will discuss with the patient the trial's purpose, procedures, risks and potential benefits, and their rights as a participant. The team will continue to update the patient on any new information that may affect their situation.

Consent will be obtained prior to any research-driven procedures. The investigator will assess the patient's capacity during his/her encounters with him or her. The investigator will give the person providing consent adequate opportunity to read the consent document before it is signed and dated.

It will be explained to prospective participants that the study involves research, the purpose of the research, the expected duration of participation, as well as the approximate number of participants to be enrolled. The study procedures, and identification of research procedures v. non-research will also be thoroughly discussed. It will be explained to participants that participation is voluntary and that the subject may discontinue at any time.

Refusal to participate or withdraw will not involve a penalty or loss of benefits to which the participant is otherwise entitled. Refusal will in no way affect the participant's future are. The participant will also be told of the possible consequences of the decision to withdraw from the research, and procedures for orderly termination of participation.

Any significant new findings developed during the course of the research that may affect the participant's willingness to continue to participate will be provided. Also explained will be anticipated circumstances under which the subject's participation may be terminated by the investigator without the participant's consent.

Prospective participants will be provided with a description of any reasonably foreseeable risks or discomforts as well as a description of any benefits to the participant or to others that might be reasons expected from the research. Alternative procedures or courses of treatment will also be thoroughly discussed.

Prospective participants will also be given detailed information describing the extent to which confidentiality of records identifying the participant will be maintained and what records may be examined by the research staff, IRBs, sponsor, their representatives, and possibly the FDA or OHRP.

Also communicated to the participant will be an explanation that emergency medical care will be arranged for a study-related illness or injury, and an explanation of whether funds are set aside to pay for this care and/or compensation, and if so by whom (e.g., sponsor, subject, insurer). The participant is told the source of the study's funding.

All participants will be told of any additional costs that may result from participation in the research.

Consent will be done in person or remotely through secured email, phone or by electronic consenting using one of the methods that is Emory LITS approved (e.g., DocuSign) when available. We will follow Emory's guidance on use of electronic informed consent.

Non-English-Speaking Participants
A certified translator/interpreter will be present during the consenting process and all questions and concerns will be answered by the treating physician.

A Short Form in that specific language will be used. A certified translator/interpreter will be present during the consenting process, and this will be documented. We will use what's available on Emory IRB website. For the languages that are not available, we will have the short form translated to that language and submit the IRB for review and approval prior to use. Process to Document Consent in Writing: Winship SOP 2.1:"Obtaining Informed consent for Interventional clinical trial" will be followed.

Participants who are not yet adults (infants, children, teenagers): N/A

Cognitively Impaired Adults: will be identified based on professional opinion of their primary physician. In this case their Legally Authorized Representative will undergo an informed consent procedure as per Emory IRB policy.

Adults Unable to Consent: permission will be obtained, via informed consent, from the Legally Authorized Representative in the next order of priority: spouse, adult child, court-appointed guardian, or attorney for health care.

Waiver or Alteration of Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception): N/A

24. Setting

The research team will conduct the research in two sites: Emory University Hospital and Universidad Central del Caribe.
Identification and recruitment of potential participants will be conducted at Emory University Hospital and Clinic, and the Winship Cancer Institute.

The research procedures will be performed at Emory University (preparation of protein samples & pharmacokinetics/pharmacodynamics), and at Central Caribbean University (Universidad Central del Caribe) (western blot analysis of protein samples in order to evaluate the level of Pyk2, FAK, MEK and Erk signaling activation).

Research procedures at the Universidad Central del Caribe will be conducted based on the Universidad Central del Caribe IRB approval, which is contingent upon and based upon the Emory IRB approval.

The Universidad Central del Caribe Human Research Subjects Protection Office and Institutional Review Board will review Emory IRB approval documentation and certify their research procedures are in line with the standards at the Universidad Central del Caribe.

25. Resources Available

Approximately 100 GBM surgeries are performed at Emory University Hospital annually. Based on our previous experience, around 20% of potential participants sign consent and participate in studies. Based on this approximation, we will need to approach approximately 60 potential participants to enroll 12 participants that complete the study as we plan. Thus, Emory University Hospital has resources for enrollment of the necessary number of participants during the year of accrual. The investigators will devote a total of approximately one calendar year to accruing cases and 18 months completing the research.

Emory Hospital has all medical or psychological resources that participants might need as a result of anticipated consequences of the human research.

All persons assisting with the research will be made familiar with the protocols and described research procedures, their duties and functions related to them.

Facilities

All necessary facilities to complete the proposed studies are available to Dr. Olson (PI) and Dr. Mayol Del Valle (CO-PI) at the Emory University Hospital in Atlanta and Dr. Kucheryavykh (collaborator) at the Universidad Central del Caribe School of Medicine (UCC) in Bayamón, Puerto Rico.

The Emory Lipidomic core has the mass-spectrometry resources required for the study and will designate appropriate personnel after consulting with the director of Emory integrated core facilities.

Dr. Olson holds and appointment of Professor of Neurosurgery at Emory University
Dr. Mayol del Valle holds an appointment of Senior Associate at Emory University

Winship Cancer Center Neurosurgery Laboratory will be used for tissue sample protein extraction with added phosphatase inhibitor, and frozen at -80 to be shipped overnight to Puerto Rico in dry ice.

Dr. Kucheryavykh holds an appointment in the Department of Biochemistry, Universidad Central del Caribe School of Medicine.

Laboratory: The laboratory is located at the department of Biochemistry of the Medical School's Main building which houses many of the shared equipment centers including the Confocal Microscope and the Optical Imaging facility. The laboratory has the basic laboratory equipment that includes centrifuges, shakers, refrigerators, –20oC freezers, fluorescent microscope Olympus 1X71, Narishige SR-5M mouse stereotaxic system for brain implantation surgeries, Amscope surgery microscope. In the core facilities, there are also an Olympus FV1000 confocal microscope, a Biorad Versadoc MP 5000 molecular imager, an applied Biosystems 7500 Real Time PCR System, fume hoods, and the cell culture room all available for common use.
The laboratory at UC Caribe is certified and inspected yearly by the office of the Dean of Administration who arranges visits with the Department of Health and Safety and the National Fire Department.

Computers: There are multiple computers available for this project. All have Internet access and are configured with standard application software for Windows systems, including Microsoft Office 11, Graph Pad Prism 8, Adobe Photoshop CS6. The gel documentation system, fluorescent microscope and other equipment have attached computers with appropriate software. Moreover, the institution provides a complete computer communications network environment with related technology and resources, including an enterprise storage and backup system for management of critical research data, email, and virus/spam protection.
Office: Dr. Kucheryavykh has her office at the Department of Biochemistry. It is equipped with a computer for data analysis and manuscript and grant preparation. Fax, copy, and printing support and convenient access to an administrative assistant for general clerical assistance and grants management support are also available in the Biochemistry Department.

26. Multi-Site Research When Emory is the Lead Site

N/A
The study is a single-site recruitment research study.

27. References

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