PennState

HRP-592 - Protocol for Human Subject Research with Use of Test Article(s)

Protocol Title:
Neuro-pharmacological properties of repurposed posaconazole in glioblastoma: A Phase 0 clinical trial

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1.0 Objectives

1.1 Study Objectives

This study will examine the neuro-pharmacological (pharmacokinetics and pharmacodynamics) profile of posaconazole (PCZ), in people with primary or recurrent high-grade gliomas (HGGs) requiring open surgical resection.

1.2 Primary Study Endpoints

Establish the neuro-pharmacokinetic profile of posaconazole, using microdialysis catheters (MDC). This will be based on the assessment of the concentration versus time curves of drug in the dialysate fluid, collected over a 24-hour period after surgery.

1.3 Secondary Study Endpoints

1. Evaluate tolerability of preoperative steady-state dosing of Posaconazole, as measured through the Grade and Frequency of adverse events, based on the CTCAE v5.0 criteria
2. Evaluate pharmacodynamics properties of posaconazole, based on
   a) Study drug's effect on Hexokinase 2 activity within resected tumor tissue, as measured using a hexokinase assay on tumor tissue following resection
   b) in resected tumor tissue.
   c) Study drug's effect on tumor proliferation (using Ki-67 proliferation index), cell death (using TUNEL staining), and angiogenesis (based on expression of VEGF) in resected tumor tissue.
   d) Correlation of posaconazole pharmacokinetic profile with that of lactate and pyruvate, using MDC. This will be assessed based on the concentration versus time profile of lactate and pyruvate in the dialysate fluid, over the same 24-hour period used to measure the concentration of drug

2.0 Background

2.1 Scientific Background and Gaps

High grade gliomas

High grade gliomas (HGGs, WHO Grade III and IV gliomas) are the most common malignant primary brain tumor in humans. Glioblastoma (GBM, WHO Grade IV glioma) is the most aggressive brain tumor, accounting for more than half of all gliomas and 17% of all primary brain and central nervous system (CNS) tumors[4]. Far from the importance of GBM being the most common human malignancy, GBM deserves attention on the basis of its universally poor prognosis.

Decades of research has demonstrated various degrees of molecular, genetic, epigenetic, and metabolic heterogeneity among tumors of the same grade and even within individual tumors [7]. This heterogeneity has significantly hampered efforts toward developing effective therapeutic strategies for GBM patients. From a metabolic perspective, it is well-known that most tumor cells preferentially convert glucose to lactate, regardless of the levels of available oxygen; this is known as aerobic glycolysis (or Warburg effect) [8, 9].

The Warburg effect and its role in HGGs

In HGGs, as with most cancers, cells have undergone effective reprogramming of their energy metabolism in order to be able to survive in hypoxic conditions [8]. Rapidly proliferating cancer cells demonstrate a preferential bias toward aerobic glycolysis over oxidative phosphorylation for supporting growth and survival in hypoxic conditions. This metabolic reprogramming is referred to as the "Warburg effect". Through this mechanism, tumor cells are able to produce large amounts of lactate while consuming very little oxygen. The dependency of cancer cell proliferation on accelerated glucose metabolism distinguishes them from their normal counterparts and could render them more vulnerable

to the disruption of glucose metabolism. Therefore, cancer cells could be selectively targeted by the disruption of intracellular glucose metabolism.

Hexokinase 2 in GBM metabolism

Hexokinases (HKs) catalyze the first committed step in glucose metabolism, which is the ATP-dependent phosphorylation of glucose to glucose-6-phosphate (G6P), which ensures a sustained concentration gradient that promotes the entry of glucose into cells. This establishes the role of HK in determining the magnitude and direction of intracellular glucose flux. The HK family of enzymes is comprised of 4 isoforms (denoted as HK1, HK2, HK3, and HK4 [11]. Among these, HK1, and HK2, are associated with mitochondria and were also implicated in cell survival [12, 13]. While HK1 is present in most human adult tissues, HK2 is only expressed in embryonic tissues or a select group of adult tissues such as adipose and skeletal/cardiac muscles [14]. What distinguishes cancer cells from normal cells is their ability to express very high levels of HK2, which promotes the accelerated flux of glucose under hypoxic conditions [15, 16]. Over-expression of HK2 has been demonstrated in many cancer types and it has been associated with a poor survival [17].

Over-expression of HK2 has been documented in GBM cells as well [18, 19]. In addition to its role in promoting the influx of glucose, HK2 also inhibits apoptosis [20]. Previously, we have shown that while HK2 is significantly upregulated in GBM, HK1 was down-regulated [21]. Our group has also previously shown that a reduction of HK2 expression (but not HK1 or HK3) *in vitro* can inhibit aerobic glycolysis, increase normal oxidative respiration, and induce apoptosis (particularly under hypoxic conditions). We have also shown that the survival of mice injected with HK2 knockdown glioma cells is extended[19] and that HK2 plays a critical role in tumor cell survival at a very early stage [21].

Through microscopic analysis of tumor cells deficient of HK2, compared with controls, our group has also shown that HK2 has the ability to alter the tumor vasculature as well [21]. Our *in vitro* work has demonstrated a significant decrease in the density of neovasculature particularly away from the central necrotic core. *In vivo*, we demonstrated that mice bearing intracranial GBM xenografts of HK2- knock down tumors had a significantly decreased vascular perfusion/permeability compared to xenografts of control tumor, based on measurement of the $K\text{-}_{trans}$ value in dynamic contrast-enhanced MR imaging (Figure 1). Furthermore, we have also shown that the absence of HK2 confers sensitivity to chemo-radiation *in vivo* (Figure 2).

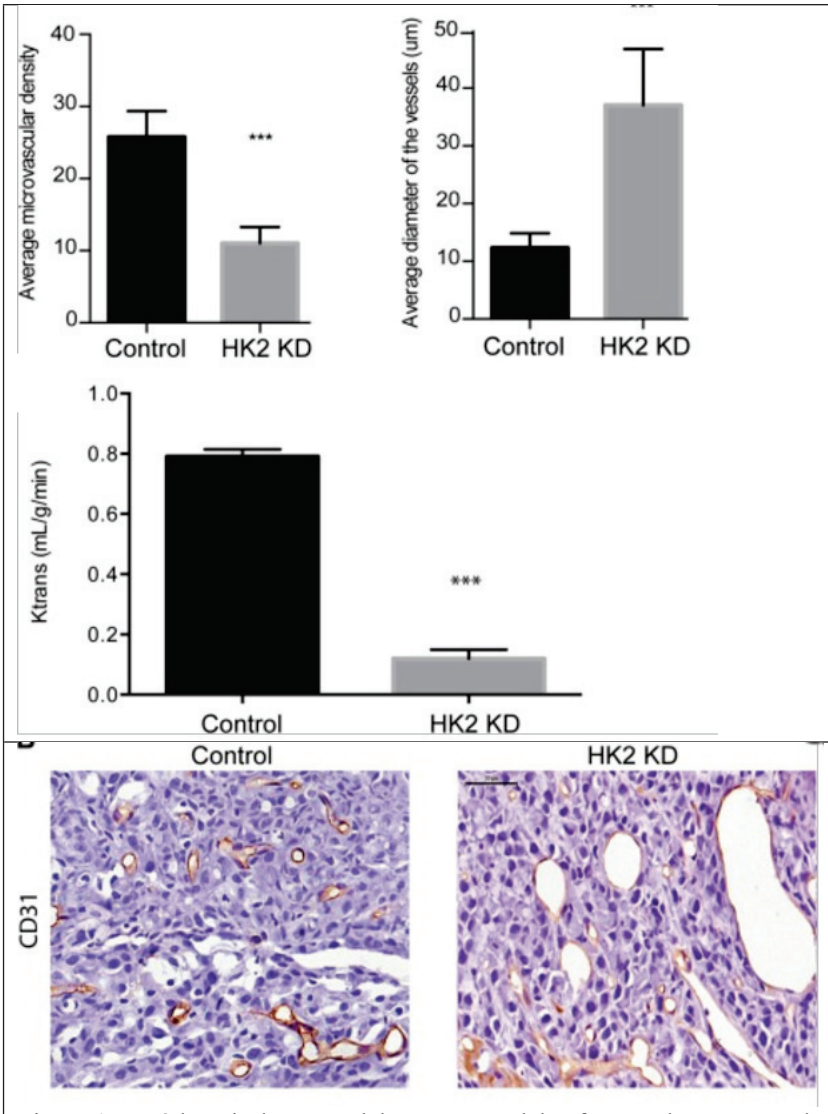


Figure 1. HK2 knock-down models mouse models of GBM demonstrate decreased microvascular density, diminished vascular permeability, and less dilated/ tortuous vessels

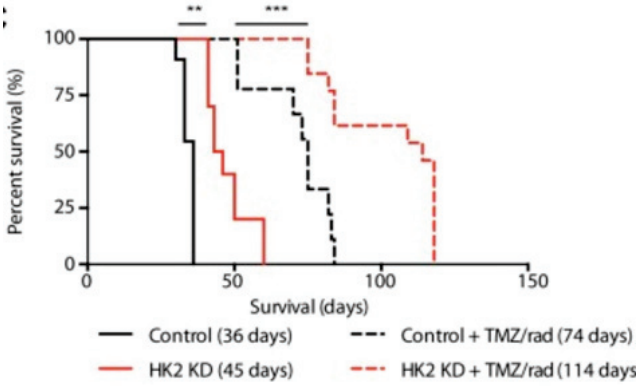


Figure 2. HK2 knock-down mouse models of GBM have a longer average survival compared to controls. This also increases the tumor cells' sensitivity to chemo-radiation (TMZ = radiation)

Therefore, it is clear that HK2 is a key driver of several oncogenic pathways in GBMs and is at least in part responsible for the tumor's increased proliferation and resistance to chemo-radiation. Currently, no direct inhibitor of HK2 has been tested in human patients with GBMs. Given the limited progress in the field of therapeutics for GBMs since the establishment of the Stupp protocol as the standard of care [22], there is a pressing need to expand the available treatment regiments for this population by

introducing new approaches or therapeutic agents. One possible means to expedite initiation of GBM clinical trials is to examine previously established drugs with a known track record of safety in humans, regardless of their intended use.

2.2 Previous Data

Identification of the potentially anti-tumor effect of azoles in patients with GBM

Using a small drug screen targeting potential HK2 regulated gene expression networks we identified the azole class of antifungals as inhibitors of tumor metabolism by reducing proliferation, lactate production, glucose uptake in GBM cells but not primary normal human astrocytes or normal neural stem cells (Figure 3) [58].

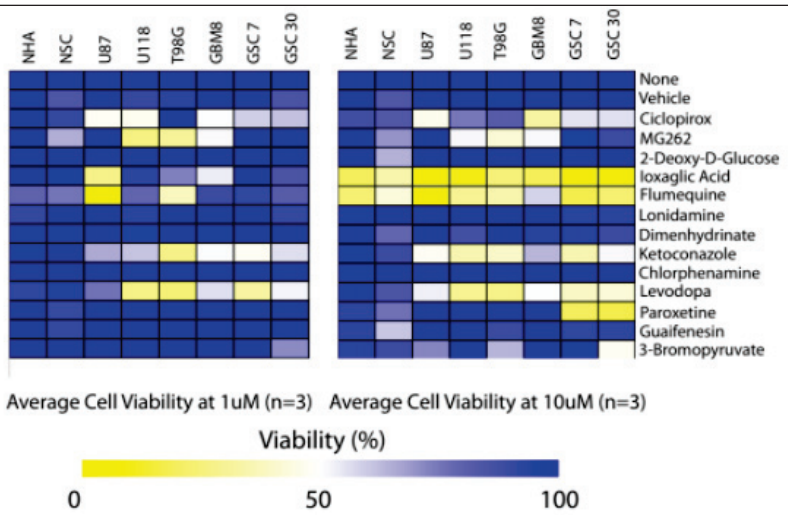


Figure 3. Small drug screen reveals several compounds that decrease the viability of GBM tumor cell lines and glioma stem cell lines, including ketoconazole

Heterozygous HXK2 Yeast

Drug	Dose (uM)	Fitness Defect	P-Value
Ketoconazole	11.8	12.9031	0.00000000035708
Lapachol	500	7.80512	0.00000000025082
Methotrexate	500	7.28509	0.000000013988
Lovastatin	62.5	4.03129	0.00010094
Berberine chloride	125	3.72723	0.00025965
Miconazole	0.050	3.01774	0.0053539
Atorvastatin	62.5	2.52484	0.0075045
Methotrexate	125	2.50201	0.0088269
Benomyl	13.8	2.17125	0.041007
5-fluorouracil	38.4	2.04176	0.024746
Cantharidin	125	2.03491	0.025107

Homozygous HXK2 Yeast

Condition1	Dose (uM)	FitnessDefect	P-value
CoCl2	1250	14.6714	< 1e-12
Clotrimazole	2	11.146	0.00000000010568
Miconazole	0.050	8.35617	0.0000000038706
Paraquat	5000	8.0611	0.0000000010666
Thiabendazole	250	7.79036	0.000000014499
Wiskostatin	29	6.70764	0.000000052684
Rapamycin	0.006	6.69979	0.000000053917
Methoxsalen	500	6.67922	0.00000021855
Hygromycin	13.75	6.5126	0.000000093713
Lovastatin	77.2	6.49968	0.00000034352

Ketoconazole and posaconazole enhance *in vitro* survival in part through down-regulation of HK2 activity

Upon identification of ketoconazole and posaconazole as potential inhibitors of HK2-regulated gene expression networks, we conducted dynamic metabolic flux analysis with 13C-labeling experiments followed by liquid chromatography-mass spectrometry (LC-MS) to assess the effect of the addition of ketoconazole or posaconazole, on glycolytic intermediates from GBM cell lines and Glioma stem cell cultures [58]. Through this approach, we demonstrated a reduction of various pro-anabolic glycolytic intermediates through the addition of these azoles (Figure 4A). This was shown to be at least in part due to the down-regulation of HK2 activity, and hence reduction of lactate production, by azoles (Figure 4 B, and C) Loss of HK2 in GBM cells dampened the effect of several azoles suggesting that the mechanism of action is mediated in part through HK2 (Figure 4D). The addition of ketoconazole or posaconazole (IC50 = 10 µM) decreased the viability of several GBM cell lines (Figure 4E) and glioma stem cells (Figure 4F) but not normal human astrocytes (NHA).

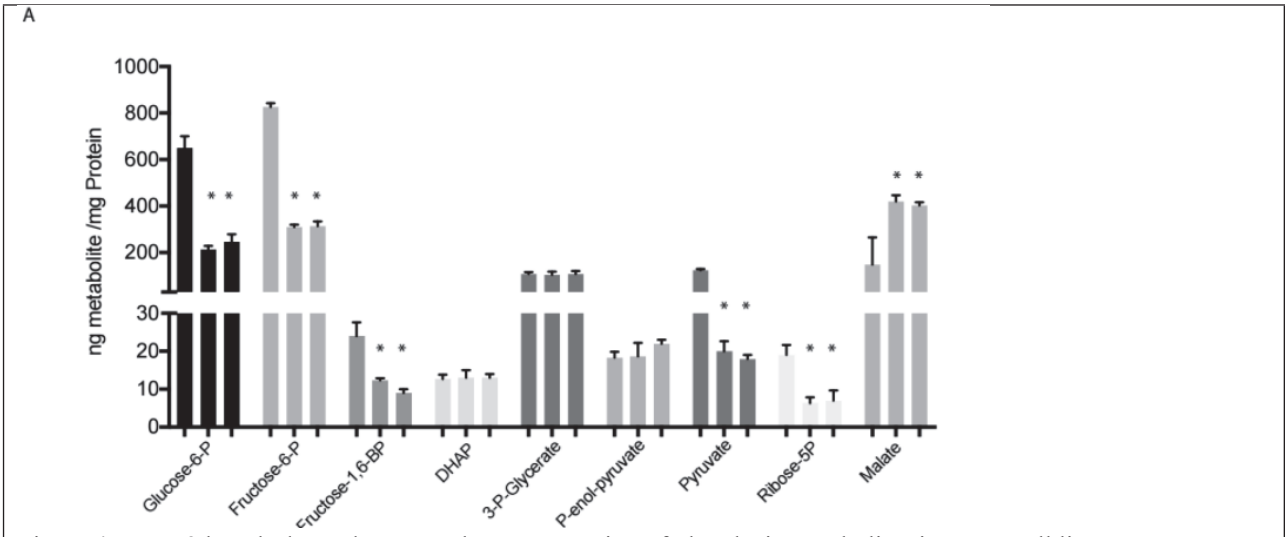


Figure 4 A. HK2 knock-down decreases the concentration of glycolysis metabolites in GBM cell lines

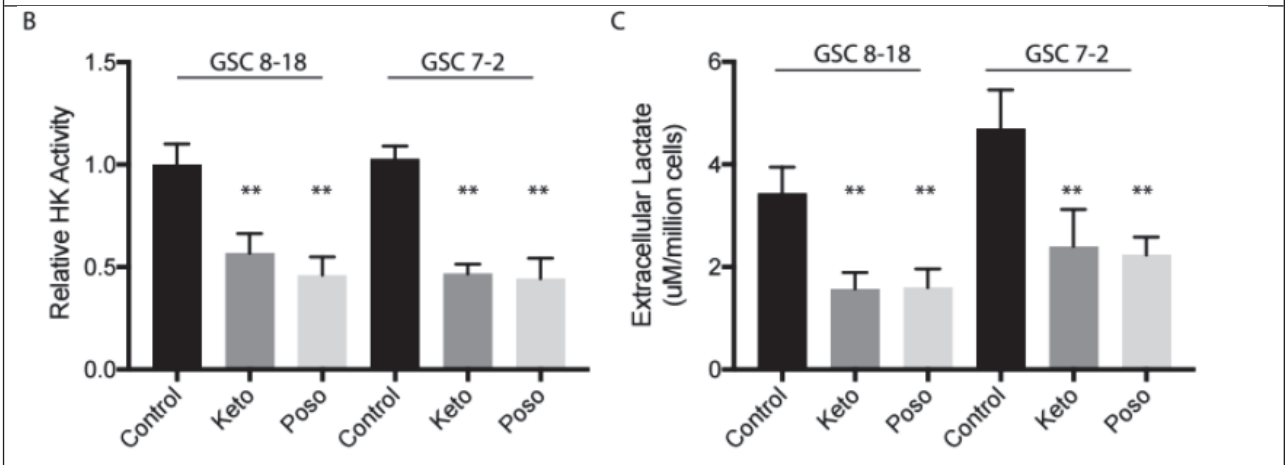


Figure 4 B, C; Ketoconazole and Posaconazole decrease the relative HK activity and, thus, extracellular lactate in glioma stem cell lines

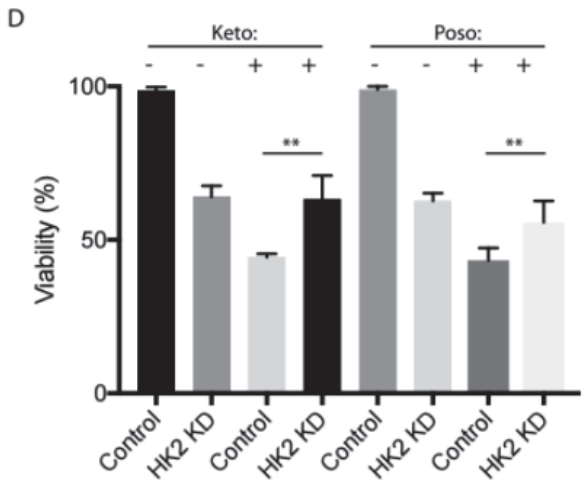


Figure 4 D. Glioma cell line viability is decreased following treatment with Ketoconazole and Posaconazole. This effect on viability is decreased in the absence of HK2 (knock-down)

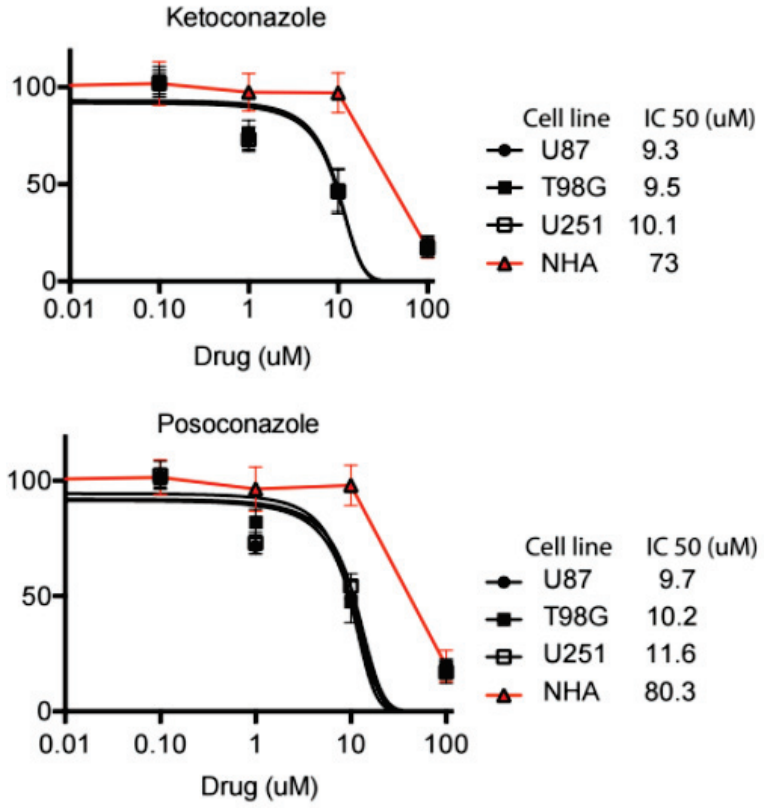


Figure 4E. Increasing concentrations of Ketoconazole and Posaconazole decrease the % viability of GBM cell lines but not normal human astrocytes (NHA).

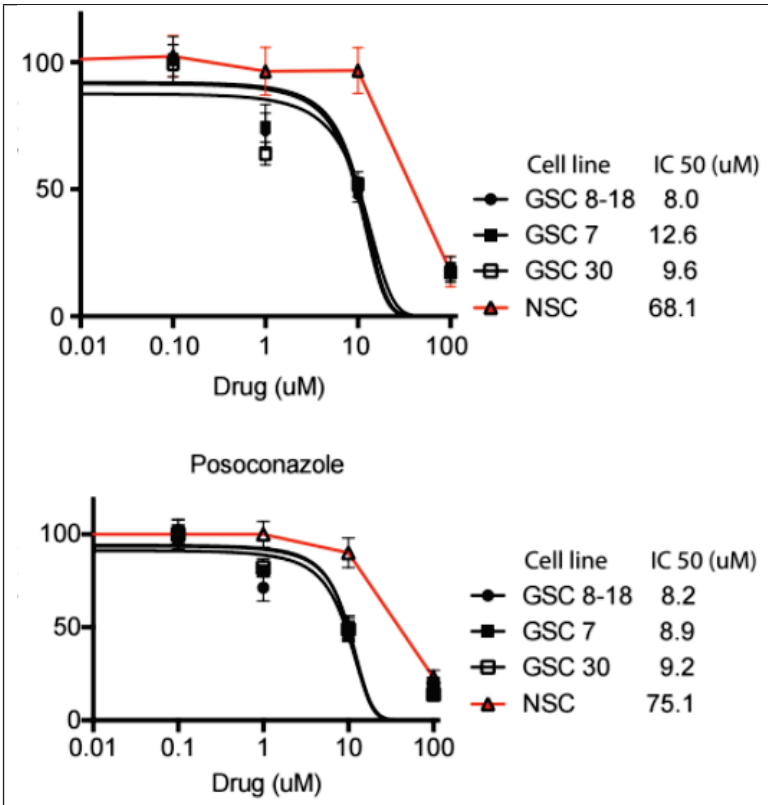


Figure 4F. Increasing concentrations of ketoconazole and posaconazole decrease the viability of glioma stem cell lines but not normal stem cell lines (NSC).

Ketoconazole and posaconazole enhance *in vivo* survival in part through down-regulation of HK2 activity

The survival of U87 xenograft mice bearing glioblastoma tumors was extended through the administration of either ketoconazole or posaconazole (25mg/kg daily) (Figure 5A) [58]. Histologically, a decrease in mitotic activity was observed following the administration of these two agents in glioma cells (Figure 5 B and C). The reduction of HK2 activity following the administration of ketoconazole or posaconazole was demonstrated *in vivo* as well, with concordant reduction of lactate production as well (Figure 5 D and E).

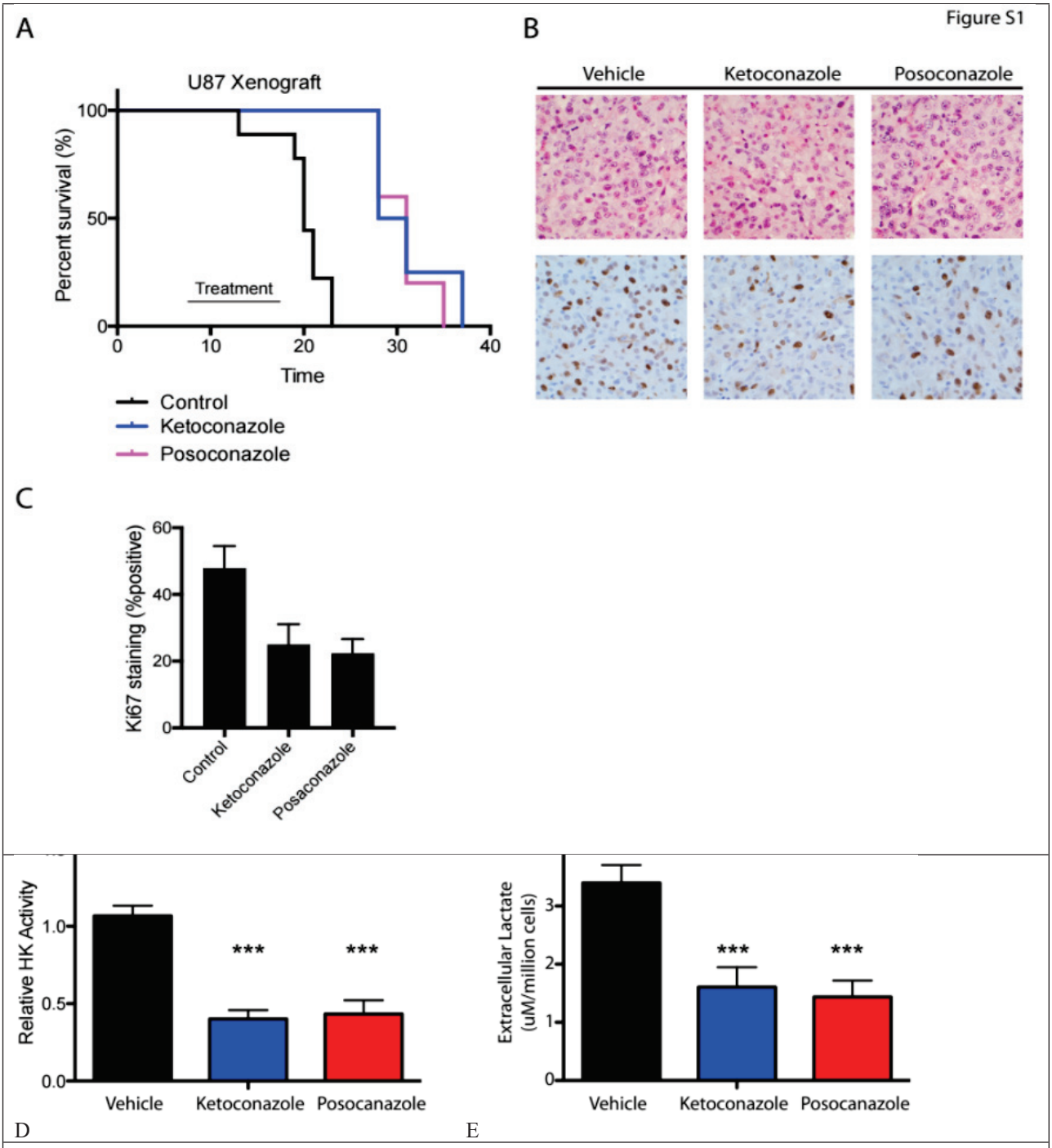


Figure 5. Enhancement of xenograft survival following the administration of ketoconazole or posaconazole (A), along with decreased mitotic activity as demonstrated through Ki67 staining (B and C). This is mediated through reduction of HK activity (D) and lactate production (E).

2.3 Study Rationale

Both ketoconazole and posaconazole are FDA-approved anti-fungal agents with a well-established side effect and safety profile. Ketoconazole and posaconazole have shown efficacy in reducing tumor cell proliferation in our *in vitro* studies. Furthermore, both have also shown efficacy, mediated at least in part through inhibition of HK2 activity, in our animal models with dosing concentration and schedules that are documented as safe in humans.[58] As a drug, posaconazole has a more predictable half-life than ketoconazole and has less off-target effects. Therefore, the proposed trial will focus on the role of posaconazole exclusively. As a first step, demonstration of adequate penetrance of study drug in brain and tumor tissue (pharmacokinetics) and biological effect (inhibition of glycolysis and subsequent tumor cell death) is necessary prior to large scale clinical studies. A total of 5 control participants will be included in this study as we specifically want to assess for pharmacodynamic differences too. The

addition of a control group to this study rather to both the studies (ketoconazole study is a separate protocol) is because we feel posaconazole may be a more promising drug for moving forward.

Plasma drug concentration measurements are an unreliable method to assess delivery of drugs across the blood-brain barrier. In contrast, intracerebral MDC monitoring allows for approximate measurements within extracellular fluid (ECF) sampling of the brain. MDC placement within the brain is not a novel technique and has been utilized routinely in the ICU setting to measure brain metabolism by sampling of ECF of traumatic brain injury patients [59-61].

MDC are now FDA-approved and are being placed routinely with intracranial pressure monitors. This method allows for continuous measurement of ECF within a tumor or normal tissue. The dialysis probe has a semipermeable membrane which is less than 1 mm in diameter into which two sections of microcatheter are fused. Previous studies have demonstrated the feasibility of keeping the catheters in place of critically injured patients for up to 2 weeks [62-64].

When placed at the time of surgical resection, the microcatheters are stereotactically implanted, placing the probe within the desired brain and/or tumor region. Externally, the catheter is connected to a syringe pump, which delivers a low flow rate (µl/min) of continuous perfusion fluid (Lactated Ringers or artificial CSF) and dialysate is collected in a microvial from the outlet tube. This sterile, single use catheter is minimally invasive and developed to achieve optimal diffusing characteristics similar to passive diffusion of a capillary blood vessel. Just as in the function of brain capillary vessel, water, inorganic ions and small organic molecules freely diffuse across the membrane of the probe, whereas proteins and protein bound compounds are impermeable. Additionally, lipophilic compounds are poorly recovered. Therefore, assessment of pharmacokinetics of drug using MDC provides valuable insight relevant to its anti-neoplastic properties.

3.0 Inclusion and Exclusion Criteria

3.1 Inclusion Criteria

Patients eligible for enrollment in this study will be male and female adults with a presumed diagnosis of primary or recurrent HGG based on standard of care imaging (contrast-enhanced MRI) and clinical presentation and for whom surgical intervention has been selected as the most appropriate treatment option by the treating physician and patient.

1. Age ≥18 years
2. Evidence of primary or recurrent HGG that in the opinion of the treating team would require surgical resection
3. Adequate liver function defined as ALT, AST, ALP within 1.5x institutional upper limit of normal (for study drug arm only)
4. Ability to swallow medication (for study drug arm only)
5. Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) for the duration of study participation (for study drug arm only)
6. Ability to understand and willingness to sign a written informed consent document
7. Be able to comply with treatment plan, study procedures and follow-up examinations

3.2 Exclusion Criteria

1. Patients may not be receiving any other investigational agents while on study
2. Patients who have known allergy to posaconazole or other azoles (for study drug arm only)
3. Patients who have previously had a severe side effect, such as agranulocytosis and neutropenia, in conjunction with previous azole class drugs for a parasitic infection (for study drug arm only)
4. Patients with a history of acute or chronic hepatitis (for study drug arm only)

5. Patients with liver enzymes (ALT, AST, ALP) >1.5x above normal range for the laboratory performing the test (for study drug arm only)
6. Patients who are taking metronidazole and cannot be safely moved to a different antibiotic greater than 7 days prior to starting posaconazole therapy (for study drug arm only)
7. Patients who are taking any anti-convulsant medication that interferes with the cytochrome P450 pathway (e.g. phenytoin, phenobarbital, carbamazepine, etc.) and who cannot be switched to alternative medications such as Keppra (levetiracetam) (for study drug arm only)
8. Uncontrolled intercurrent illness such as chronic hepatitis, acute hepatitis, or psychiatric illness/social situation that would limit compliance with study requirements (for study drug arm only)
9. Patients with a history of Addison's disease or other forms of adrenal insufficiency (for study drug arm only)
10. Patient with little or no stomach acid production (achlorhydria) (for study drug arm only)
11. Pregnant and breastfeeding women)
12. Patients with a history of any medical or psychiatric condition or laboratory abnormality that in the opinion of the investigator may increase the risks associated with the study participation or investigational product administration or may interfere with the interpretation of the results.
13. Patients who are not available for follow-up assessments or unable to comply with study requirements
14. Patients who are currently taking medications that induce the metabolism of posaconazole, such as isoniazid, nevirapine, rifamycins (such as rifabutin, rifampin), or St. John's wort and cannot be safely discontinued off of them for the duration of the trial (for study drug arm only).
15. Patients who are currently taking medications for which the metabolism may be affected by posaconazole, which include but are not limited to: benzodiazepines (such as alprazolam, midazolam, triazolam), domperidone, eletriptan, eplerenone, ergot drugs (such asergotamine), nisoldipine, drugs used to treat erectile dysfunction-ED or pulmonary hypertension (such as sildenafil, tadalafil), some drugs used to treat seizures (such as carbamazepine, phenytoin), some statin drugs (such as atorvastatin, lovastatin, simvastatin) (for study drug arm only)
16. Patients who are non-English speakers
17. Patients who are not capable of understanding the consent form and would need a legally authorized representative

3.3 Early Withdrawal of Subjects

3.3.1 Criteria for removal from study

A participant should be withdrawn from the trial treatment if, in the opinion of the PI, it is medically necessary, or if it is the wish of the participant. If a participant does not return for a scheduled visit, every effort will be made to contact the participant. In any circumstance, every effort will be made to document participant outcome, if possible.

Participants should be removed from therapy if any of the following occurs:

- Adverse event: The occurrence of unacceptable toxicity indicating the need for cessation of treatment
- The physician feels it is in the best interest of the patient to stop treatment
- The participant desired to withdraw from further participation in the study in the absence of an investigator-determined medical need to withdraw. If a reason for withdrawal is given, it should be recorded in the case report form
- Protocol violation: The participant ‘s findings or conduct failed to meet the protocol entry criteria or failed to adhere to the protocol requirements (e.g. study drug compliance, returning for the specified number of visits)
- Participant is lost to follow-up. If a participant does not return for scheduled visits, every effort should be made to re-establish contact. In any circumstance, every effort should be made to document participant outcome, if possible

- Participant becomes pregnant
- Termination of the study

The reason and date of discontinuation are to be documented in the participant 's medical record and in the case report form. All participants who receive one or more doses of study treatment should be included in any safety analysis. The PI may discontinue the trial at any time. Reasons for early trial discontinuation may include, but are not limited to, unacceptable toxicity of study treatment, a request to discontinue the trial from a regulatory authority, or poor enrollment.

3.3.2 Follow-up for withdrawn subjects

The PI will complete all end of treatment procedures when a participant withdraws from treatment. All participants who discontinue treatment secondary to an AE will be followed until resolution, stabilization or return to a baseline condition. Participants who fail to complete treatment for reasons other than AE or unacceptable toxicity may be replaced.

4.0 Recruitment Methods

4.1 Identification of subjects

Potential subjects will be identified by the principal investigator when they attend their clinic appointment or in the emergency department if no urgent surgery or biopsy indicated.

4.2 Recruitment process

4.2.1 How potential subjects will be recruited.

Potential subjects will be approached by the physicians in clinic during their regularly scheduled clinic visit or in the emergency department if no urgent surgery or biopsy indicated. If at that time the physician feels that patient would be a good fit for the study, she/he will then introduce the patient to the study to gauge interest. The study will be described to the patient and they will be given a chance to ask any additional questions. If the patient agrees to volunteer for the study, informed consent will be obtained.

4.2.2 Where potential subjects will be recruited.

Patients will be recruited in the clinic or in the emergency department if no urgent surgery or biopsy indicated.

4.2.3 When potential subjects will be recruited.

Patients will be recruited prior to their scheduled biopsy or surgery.

4.2.4 Describe the eligibility screening process and indicate whether the screening process will occur before or after obtaining informed consent. Screening begins when the investigator obtains information about or from a prospective participant in order to determine their eligibility. In some studies, these procedures may not take place unless HIPAA Authorization is obtained OR a waiver of HIPAA Authorization when applicable for the screening procedures is approved by the IRB.

Screening of subjects at is expected prior to obtaining consent to determine potential eligibility.

Once informed consent is obtained, patients will undergo additional screening to determine if they are eligible to participate in the study based on the inclusion/exclusion criteria. If a participant is ineligible for the study drug arm, they will first be considered for candidacy in the ketoconazole study (STUDY00014115). If enrollment in the ketoconazole study is already completed or if the participant is not a candidate for ketoconazole either, then they will be considered for possible candidacy in the control arm of this study.

5.0 Consent Process and Documentation

The PI and/or delegated research staff will obtain informed consent from each participant enrolled in the study, in accordance with FDA regulations and will adhere to Good Clinical Practices and to the ethical principles that have their origin in the Declaration of Helsinki. Consent will only be obtained under circumstances that provide the prospective subject sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. All personnel involved with recruitment and obtaining informed consent will have completed training in the protection of human subjects in research and been approved by the IRB to participate in the study. Prior to the beginning of the trial, the PI will have the IRB approval of the written informed consent form and any other written information provided to subjects. Subjects will not be consented until final IRB approval of these documents is received.

5.1 Consent Process:

Check all applicable boxes below:

☒ **Informed consent will be sought and documented with a written consent form *[Complete Sections 5.2 and 5.6]***

☐ **Implied or verbal consent will be obtained – subjects will not sign a consent form (waiver of written documentation of consent) *[Complete Sections 5.2, 5.3 and 5.6]***

☐ **Informed consent will be sought but some of the elements of informed consent will be omitted or altered (e.g., deception). *[Complete section 5.2, 5.4 and 5.6]***

☐ **Informed consent will not be obtained – request to completely waive the informed consent requirement. *[Complete Section 5.5]***

5.2 Obtaining Informed Consent

As part of the initial neuro exam, it will be determined if the individual is capable of signing and understanding the consent and research study. A physician/investigator will determine the capability of any potential research subject's ability to consent, through standard neuro and cognitive testing completed initially upon arrival at our institution.

After it has been determined that an individual is eligible to participate, the study protocol, including all risks and potential benefits and investigational nature, will be explained to the participant by the PI and/or delegated research personnel in a private setting with respect to the potential subject's privacy. The PI or delegated research staff will review and discuss details of the research study using the consent form as a guide. All basic elements of the consent form document, consent addendums and any additional relevant information will be presented in detail to the prospective subject. The information given to the subject will be in a language understandable to the subject. No informed consent, whether oral or written, will include any exculpatory language through which the subject is made to waive or appear to waive any of the subject's legal rights, or releases or appears to release the investigator, the sponsor, the institution or its agents from liability for negligence. The information presented will not include complex, technical, highly specialized language or medical jargon that would not be understandable to potential subject. The PI and delegated research staff will not coerce or unduly influence a subject to participate or to continue to participate in a trial.

Before informed consent may be obtained, the PI or delegated research staff will provide the subject ample time and opportunity to read, inquire about the details of the study and to decide whether or not to participate. All questions about the study will be answered to the satisfaction of the subject by the PI or delegated research staff.

The PI or delegated research staff will obtain informed consent which will be documented by the use of a written consent form, approved by the IRB and signed and dated in pen, by the subject prior administering the first dose of the study medicine. This form will also be signed and dated in pen by the person obtaining consent and if necessary a witness to the consent process and maintained in an individual subject research binder.

All blanks on the consent form, consent addendum forms (if applicable) for subject name, subject initials, dates, signatures, yes/no check boxes for optional research procedures must be completed by the subject themselves if previously approved by the IRB. Delegated research staff will not complete these blanks for the subject.

The PI or delegated research staff will ensure that the subject expresses understanding of information presented on the clinical trial, that participation is voluntary, and that the subject can withdraw at any time without penalty or effects to their medical care. They will ensure that all of the subject‘s questions have been answered. Participants will also be made aware that by signing the consent form their personal health information and research records may be audited/reviewed by authorized study personnel.

By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to and understood by the subject, and that informed consent was freely given by the subject. The PI or delegated research staff will give a copy of all signed and completed forms to the subject.

The PI will ensure that the subject understands that in order to participate on the clinical trial the subject must be eligible per the protocol‘s inclusion and exclusion criteria.

The informed consent document will clearly describe the potential risks and benefits of the trial, and each prospective participant must be given adequate time to discuss the trial with the PI or delegated staff and to decide whether or not to participate. The informed consent will be approved by the IRB prior to being presented to a potential patient.

Telephone Consent Process
The physician/study coordinator will talk to potential participant at their initial visit. If they express interest, we will send a copy of the informed consent form to them ahead of the telephone call for them to review. We will arrange to contact them at an agreed upon date/time.

The study coordinator will conduct the telephone consent discussion with the potential participant. A witness will be present with the study team member during the telephone consent discussion. A review of the consent will be completed. All questions and concerns will be answered prior to the end of the discussion. Time will be given for the potential participant to respond to the consent process, and give a positive response, or decline to enroll in the study.

If the potential participant says yes, they will be asked to sign, date, time, and print their name on the consent form, as we sign the telephone consent signature page at our site.

They will be asked to return the signed consent form to the research site via email, phone text message with an attached picture of the signed consent page or fax. In order to return the signed document, a telephone number, email address or fax number will be given to the potential participant.

Once received, we will place our signed telephone consent signature page with the participant’s signed consent. A copy of the entire consent will be sent by mail, email or fax to the participant.

The phone consent procedure will be included in the note to file used to document the informed consent process in the research record or medical record. A copy of the signed consent form will be placed in the medical record.

A reminder of possible loss of confidentiality may occur with email, telephone or fax, but we will be careful to give and review the telephone or fax number, and/or email address of the subject.

5.3 Waiver of Written Documentation of Consent

Not applicable

5.3.1 Indicate which of the following conditions applies to this research:

☐ The research presents no more that minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context.

OR

☐ The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality. Each subject will be asked whether the subject wants documentation linking the subject with the research, and the subject's wishes will govern.

OR

☐ If the subjects or legally authorized representatives are members of a distinct cultural group or community in which signing forms is not the norm, that the research presents no more than minimal risk of harm to subjects and provided there is an appropriate alternative mechanism for documenting that informed consent was obtained.

Describe the alternative mechanism for documenting that informed consent was obtained:

Not applicable

5.3.2 Indicate what materials, if any, will be used to inform potential subjects about the research (e.g., a letter accompanying a questionnaire, verbal script, implied consent form, or summary explanation of the research)

Not applicable

5.4 Informed consent will be sought but some of the elements of informed consent will be omitted or altered (e.g., deception).

Not applicable

5.4.1 Indicate the elements of informed consent to be omitted or altered

Not applicable

5.4.2 Indicate why the research could not practicably be carried out without the omission or alteration of consent elements

Not applicable

5.4.3 Describe why the research involves no more than minimal risk to subjects.

Not applicable

5.4.4 Describe why the alteration/omission will not adversely affect the rights and welfare of subjects.

Not applicable

5.4.5 If the research involves using identifiable private information or identifiable biospecimens, describe why the research could not be practicably be carried out without using such information or biospecimens in an identifiable format.

Not applicable

5.4.6 Debriefing

Not applicable

5.5 Informed consent will not be obtained – request to completely waive the informed consent requirement

Not applicable

5.5.1 Indicate why the research could not practicably be carried out without the waiver of consent

Not applicable

5.5.2 Describe why the research involves no more than minimal risk to subjects.

Not applicable

5.5.3 Describe why the alteration/omission will not adversely affect the rights and welfare of subjects.

Not applicable

5.5.4 If the research involves using identifiable private information or identifiable biospecimens, describe why the research could not be practicably be carried out without using such information or biospecimens in an identifiable format.

Not applicable

5.5.5 Additional pertinent information after participation

Not applicable

5.6 Consent – Other Considerations

5.6.1 Non-English-Speaking Subjects

Non-English speaking subjects will not be eligible for the trial. We do not believe that excluding these patients from this pilot study will impact the study results, as our Neuro-oncology Department does not see many non-English speakers. If the study moves into the next phase, we will look at including non-English speakers.

5.6.2 Cognitively Impaired Adults

Cognitively impaired adults will not be consented for this pilot study as patients are required to be able to understand the consent form. We do not believe that excluding these patients from this pilot study will impact the study results, as most of the patients our Neuro-oncology Department sees for biopsy or surgery are not cognitively impaired. If the study moves into the next phase, we will look at including cognitively impaired adults.

5.6.2.1 Capability of Providing Consent

Not applicable

5.6.2.2 Adults Unable to Consent

Not applicable

5.6.2.3 Assent of Adults Unable to Consent

Not applicable

5.6.3.1 Parental Permission

Not applicable

5.6.3.2 Assent of subjects who are not yet adults

Not applicable

6.0 HIPAA Research Authorization and/or Waiver or Alteration of Authorization

6.1 Authorization and/or Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

Check all that apply:

☐ **Not applicable, no identifiable protected health information (PHI) is accessed, used or disclosed in this study.** *[Mark all parts of sections 6.2 and 6.3 as not applicable]*

☒ **Authorization will be obtained and documented as part of the consent process.** *[If this is the only box checked, mark sections 6.2 and 6.3 as not applicable]*

☒ **Partial waiver is requested for recruitment purposes only (Check this box if patients' medical records will be accessed to determine eligibility before consent/authorization has been obtained).** *[Complete all parts of sections 6.2 and 6.3]*

☐ **Full waiver is requested for entire research study (e.g., medical record review studies).** *[Complete all parts of sections 6.2 and 6.3]*

☐ **Alteration is requested to waive requirement for written documentation of authorization (verbal authorization will be obtained).** *[Complete all parts of sections 6.2 and 6.3]*

6.2 Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

6.2.1 Access, use or disclosure of PHI representing no more than a minimal risk to the privacy of the individual

6.2.1.1 Plan to protect PHI from improper use or disclosure

Information is included in the "Confidentiality, Privacy and Data Management" section of this protocol.

6.2.1.2 Plan to destroy identifiers or a justification for retaining identifiers

Minimal screening activities prior to consent may include accessing patient charts to confirm eligibility. No identifiers will be retained for any patient who does not meet eligibility requirements or provide consent to participate in the study.

6.2.2 Explanation for why the research could not practicably be conducted without access to and use of PHI

Potential study participants are expected to be identified by study clinical investigators, as part of their routine clinical duties. Once identified, other members of the study team may access the patient chart to confirm eligibility and/or identity of the subject.

6.2.3 Explanation for why the research could not practicably be conducted without the waiver or alteration of authorization

Access to the patient chart prior to consent is required in order to adequately identify subjects and avoid unnecessary consent discussions (ineligible patients).

6.3 Waiver or alteration of authorization statements of agreement

Protected health information obtained as part of this research will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study, or for other permitted uses and disclosures according to federal regulations.

The research team will collect only information essential to the study and in accord with the 'Minimum Necessary' standard (information reasonably necessary to accomplish the objectives of the research) per federal regulations.

Access to the information will be limited, to the greatest extent possible, within the research team. All disclosures or releases of identifiable information granted under this waiver will be accounted for and documented.

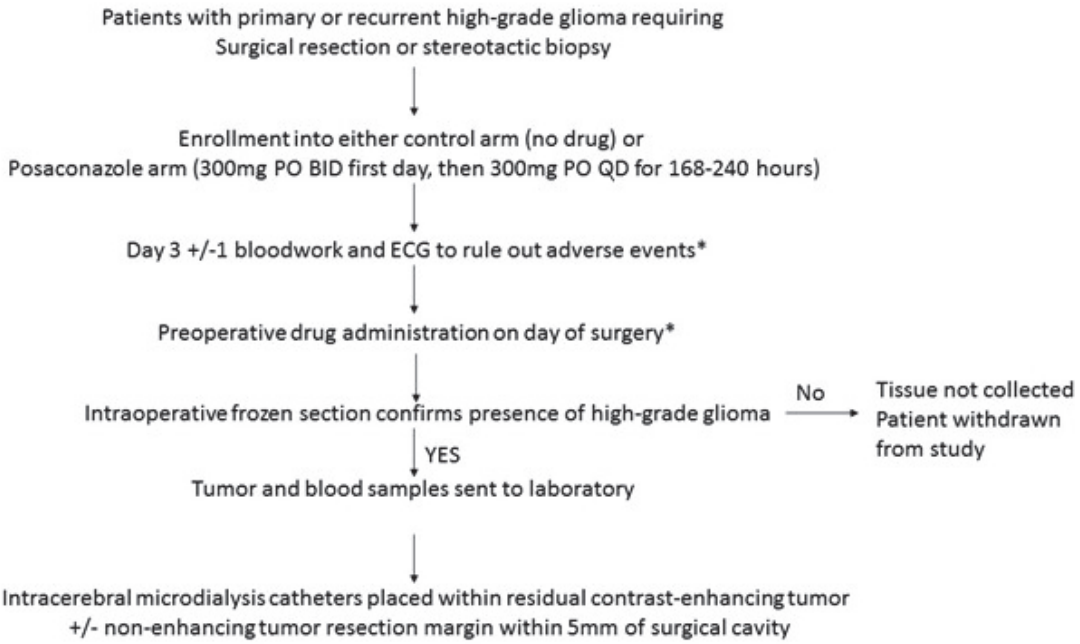
7.0 Study Design and Procedures

7.1 Study Design

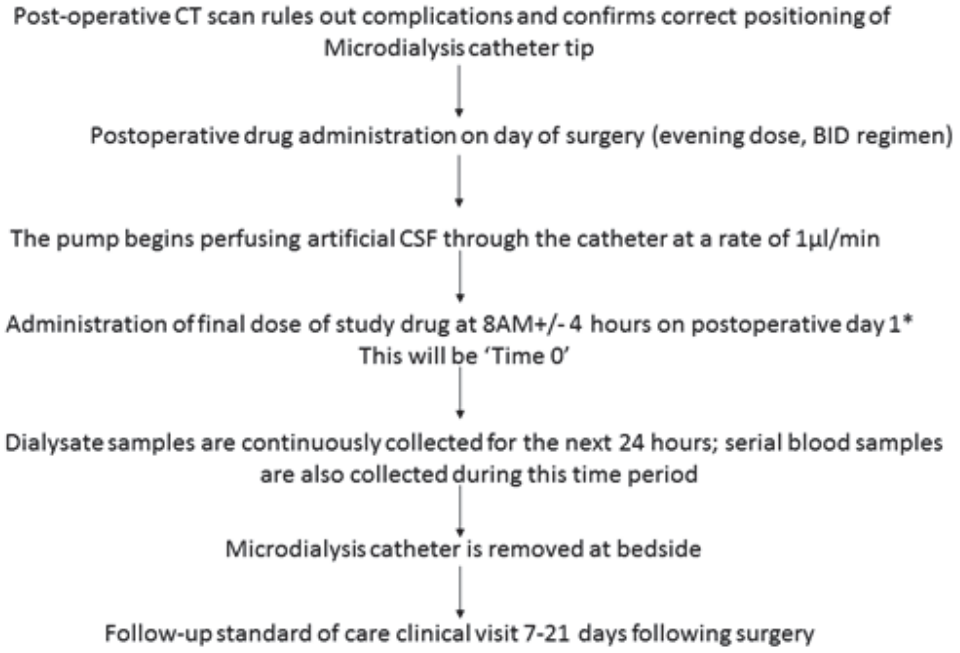
This will be a single-center, open label, non-randomized Phase 0 feasibility trial to determine the neuro-pharmacokinetic/pharmacodynamic profile of posaconazole for HGGs. We will enroll 10 (5 for study group and 5 for control group) evaluable patients over 12 months with recent MRI findings suggestive of

high grade gliomas with need for surgical resection. Our historical surgical volume supports the feasibility of this proposal.

Study Schema



* Study drug arm only



7.2 Study Procedures

If the patient appears to be eligible for the study, based on the initial evaluation, the study will be described to the patient and they will be given a chance to ask any additional questions. If the patient agrees to volunteer for the study, informed consent will be obtained.

If the patient is not eligible for enrollment in the study drug arm (e.g. contraindications to posaconazole, possible drug-drug interactions), they will be assessed for candidacy in the ketoconazole study, which has a separate IRB-approved protocol (IRB# STUDY00014115). If enrollment in the ketoconazole study is already completed or if the participant is not a candidate for ketoconazole either, then they will be considered for possible candidacy in the control arm. If, in this scenario, 5 participants have already been enrolled in the control arm, then the patient will not be a candidate for this study.

If the subject is deemed ineligible or wishes to not proceed with enrollment, then the delegated research coordinator or specialist will document the reason the subject was not enrolled in the trial and will update the site screening log appropriately.

A screening log will be used to store information from all potential participants screened. Information collected on the screening log will include participant initials, demographics (gender, race), date of consent, checkbox to note if signed informed consent given to subject, checkbox to indicate if subject enrolled, and reason for exclusion if subject not enrolled. The screening log will be kept in the regulatory binder by the study principal investigator to monitor study accrual.

7.2.1 Visit 1 (Screening and baseline)

Once informed consent has been obtained, the following are performed as **routine (standard) care** or data collected from the EMR if they were performed within 31 days of consent:

- Demographics.
- CBC and Comprehensive Metabolic Panel (which includes electrolytes, renal function and liver function) (approximately 2 teaspoons of blood).
- Medical history.
- Neurological examination.
- Baseline MRI Brain +/- Gadolinium contrast scan will be reviewed.
- Concomitant medications are reviewed.
- A 12-lead ECG.
- If a female of childbearing age, a pregnancy test will be performed.

Blood will be drawn for Comprehensive Metabolic Panel (CMP) **for the study drug group only** (approximately 2 teaspoons of blood). Patients will wait at the clinic or emergency department if no urgent surgery or biopsy is indicated until these results are available (usually within 4 hours).

If the participant's routine tests show that they are eligible and their liver function tests are acceptable, they will be able to continue in the study.

If the patient is pregnant, confirmed by the pregnancy test, the medical team can take appropriate steps to ensure the safety of both the mother and the fetus, and the patient will not be able to continue study and will be considered a screen fail.

If any of routine tests or liver function tests are outside the acceptable ranges for the study, they will be assessed for candidacy in the ketoconazole study, which has a separate IRB-approved protocol (IRB# STUDY00014115). If enrollment in the ketoconazole study is already completed or if the participant is not a candidate for ketoconazole either, then they will be considered for possible candidacy in the control arm. If, in this scenario, 5 participants have already been

enrolled in the control arm then the patient will not be a candidate for this study and they will be considered a screen fail for this study. The study doctor will follow-up regarding any abnormal results for clinical purposes.

Control group

Participants will first be considered for enrolment in the posaconazole arm. If there are contraindications to posaconazole, or if enrolment in posaconazole arm has been completed, then subjects will be enrolled in the ketoconazole study, which has a separate IRB-approved protocol (IRB# STUDY00014115). If enrollment in the ketoconazole study is already completed or if the participant is not a candidate for ketoconazole either, then they will be considered for possible candidacy in the control arm.

Study drug group

Participants will be given 12 days' worth of the study drug (pills) and verbally instructed how and when to take them. The prescription bottle will have a label with this information on it as well.

The timing of study drug administration will be based on the scheduled date and time of biopsy or surgery, which will occur within 7-10 days of Visit 1. The latter parameter is based on the drug half-life, as follows:

- Participants will be taking posaconazole as 300mg PO BID in the first day, followed by 300mg PO once daily thereafter.
- The mean elimination half-life of posaconazole is 26-31 hours (product monograph). Therefore, posaconazole will reach steady state at approximately 130-155 hours following the first dose (5 half-lives). As such, we will work backwards from the scheduled date and time of biopsy or surgery such that participants in the posaconazole arm will receive drug for 168-240 hours. The upper range of the dosing (240 hours) is to minimize potentially unnecessary exposure to drug while accounting for variability in half-life measurements.

In order to maintain steady-state concentrations, study drug will be administered at the following times as well:

1. Pre-operatively on the morning of the planned surgical intervention
2. On the morning of postoperative day 1, at 8:00AM +/- 4 hours *****

*Only if postoperative CT rules out complications and the MDC(s) are correctly positioned.

Concurrent treatment considerations and restrictions:

- Patients with HGGs will often have peri-tumoral edema for which corticosteroids are often effective. Given the influence of posaconazole on suppressing adrenal function, this exogenous steroid will also help to replace affected adrenal function and, therefore, concomitant use will be allowed. The corticosteroid of choice is dexamethasone, at a dose of 4mg PO QID. Stress dosing at time of biopsy or surgery will be at discretion of surgical and anesthesia teams.
- Due to the possible adverse effect of corticosteroids on the protective gastric lining, patients are often required to concomitantly take H2 blockers such as ranitidine (150mg po BID) during their course of corticosteroids. This could adversely affect the absorption of posaconazole. Therefore, patients will take posaconazole at least 2 hours before taking ranitidine.
- Other drugs that decrease the amount of stomach acid include over-the-counter antacids, proton pump inhibitors, sucralfate. Drugs that slow down gut movement

include anticholinergics such as dicyclomine, propantheline. Patients taking either class of drugs will be advised to take posaconazole at least 2 hours before.
- Patients will be asked to avoid alcohol for at least 3 days after finishing the drug.

7.2.2 Visit 2 (3 days +/-2 days after starting the study drug)

This visit is ONLY for the study drug arm. Participants will return to the clinic for a **research-only visit**. At this visit, the following will be performed:

- Collection of adverse events.
- A 12-lead ECG to rule out QTc prolongation
- Comprehensive Metabolic Panel (which includes electrolytes, renal function and liver function). Lab values within 1.5x institutional upper limit of normal are acceptable and not considered adverse events.

7.2.3 Visit 3 (Day of biopsy or surgery)

Control group

The following will be performed for the **research only**:

- Collection of adverse events.

Study drug group

Participants will bring their study drug prescription bottle with them.

The following will be performed for the **research only**:

- Patient will take study drug at 8am (+/- 4 hours)
- Collection of adverse events.
- Study Medication Compliance and checking of medication diary.
- Record date and time of study medication of this day for the research records and to confirm it was taken in the morning.
- If HGG diagnosis verified, record date and time of tissue sample collection, blood sample collection and microdialysis catheter placement.

Intra-operative procedure

Assessments common to both control and study drug groups

After surgical resection, frozen pathology will be reviewed to verify the diagnosis of HGG. Once pathology has been verified, and it is determined there is adequate tissue for clinical diagnosis, we will take our research sample will be taken and prepared for assessment of intra-tumoral drug concentration (study drug arm only) and assessment of HK2 activity, downstream glycolysis metabolites, tumor cell proliferation, and angiogenesis.

Where an open surgical resection has been planned, one (1) tissue sample will be from the center of the tumor and one (1) from the tumor capsule and snap-frozen immediately in the operating room (<15 minutes). No individual sample will be greater than 1cm x 1cm. These will then be divided for individual research-specific analyses.

As noted below for postoperative plasma collection schedule, blood samples will be obtained at the time of tumor sampling as well to determine and compare tumor: blood drug concentration ratios. The exact time of tumor resection and blood sampling will be recorded.

For patients in whom the intraoperative diagnosis is not consistent with HGG, no additional blood will be drawn and the patient will not be able to continue study and will be considered a

screen fail. Furthermore, no MDC will be inserted. Surgical procedures necessary for the management for the particular diagnosis will be undertaken as per standard protocol for the surgeon.

Upon satisfactory resection of tumor bulk, the neurosurgeon will place two CMA 70 MDCs with Gold Tip - (ref. no. P000050, CMA, Solna, Sweden) with a membrane length of 10mm, and shaft length 100mm - in accordance with pre-op MRI. For patients with a subtotal resection of enhancing tumor, one MDC will be placed in a contrast enhancing area of the tumor and another will be placed in a non-contrast enhancing region (normal brain) within 5mm of tumor resection cavity. For patients with a complete resection of contrast-enhancing tumor, only one MDC will be placed in the non-enhancing tumor resection boundary, within 5mm of resection cavity. Ultimately, the decision on number of catheters inserted will be at the discretion of the surgeon. Upon completion of the surgical procedure, the wound will then be irrigated and closed in standard fashion and lastly the catheter will be anchored to the scalp with sutures and sterile dressings.

7.2.4 Visit 4 (Post-operative, Day 0)

Control group

After biopsy or surgery is completed, patients will have the following as **routine (standard care)**:

- Computed tomography (CT) of the head.
- Neurological examination.

The following will be performed for the **research only**:

- Collection of adverse events experienced during biopsy or surgery as they relate to insertion of microdialysis catheter, if any.

Study drug group

After biopsy or surgery is completed, patients will have the following as **routine (standard care)**:

- Computed tomography (CT) of the head.
- Neurological examination.

The following will be performed for the **research only**:

- Collection of adverse events experienced during biopsy or surgery as they relate to insertion of microdialysis catheter.

A postoperative CT scan will be performed, as part of the standard of care to rule out immediate postoperative complications such as hemorrhage at catheter tip or misplaced catheter. Other surgical changes will not be considered to be related to the catheter insertion. This will also help confirm the position of the MDC tip: the postoperative CT image is fused with preoperative contrast-enhanced MRI to confirm position within enhancing vs non-enhancing regions. It should be performed after the surgery, prior to the dialysate sample collection the following morning. Per neurosurgical discretion, if the catheter appears malpositioned on imaging or significant tumor bed hemorrhage is present, the microdialysis studies will not be performed. The CT study will be considered abnormal for study purposes if related to the catheter insertion.

7.2.5 Visit 5 (Post-operative, Day 1)

Control group

The following will be performed as **routine (standard care)**:

- Neurological examination.

The following will be performed for the **research only**:

- Dialysate collection and blood sample collection.
- Collection of adverse events.

Study drug group

The following will be performed as **routine (standard care)**:

- Neurological examination.

The following will be performed for the **research only**:

- Patient will take study medication at 8am +/- 4 hours and then study medication will be discontinued.
- Comprehensive Metabolic Panel (which includes electrolytes, renal function and liver function) (approximately 1 teaspoon of blood). Lab values within 1.5x institutional upper limit of normal are acceptable and not considered adverse events.
- Dialysate and blood sample collection.
- Collection of adverse events.

Microdialysis collection/monitoring

After the imaging confirms proper placement of the MDC tip, the inlet tubing will be connected to a portable syringe pump to perfuse artificial CSF (Perfusion Fluid CNS, ref. no. P000151, CMA, Solna, Sweden) at a rate of 1.0 µl/min. The inlet tubing of the catheter will be connected to a CMA 107 Microdialysis Pump (ref. no. P000127, CMA, Solna Sweden). A microvial will be connected at the end of the outlet tubing to collect perfusate. Concurrently, a plastic microvial (Ref. No. P000001, CMA, Solna, Sweden) will be placed in the vial holder at the end of the outlet tubing to collect dialysate. This microvial can hold up to 200µL of fluid. It will need to be replaced with a new one every 3 hours. During daytime work week hours, trained research coordinators, in coordination with nursing team will collect dialysate and replace the microvials. At all other times, these tasks will be performed by the study PI, Dr Mansouri.

Dialysate sample collection will begin in the morning of postoperative day 1. If the participant was able to tolerate the AM dose of study drug (study drug group only), sample collection will begin immediately after drug administration. Otherwise, sample collection will begin within the same allotted time period (i.e. 8AM +/- 4 hours). For the control group, sample collection will be at 8 AM +/- 4 hours. The initial sample will be labeled as the 'Time 0' sample. Additional samples will be collected at 15 and 30 minutes, along with 1, 2, 4, 6, 8, and 24 hours after 'Time 0'.

Microvials containing the dialysate should be stored in an ultralow temperature freezer (≤-70°C) until they can be batch analyzed by LC/MS/MS.

Plasma collection

As part of the standard of care, patients will have an arterial line placed at the time of biopsy or surgery, which is usually discontinued 24-48 hours postoperatively, upon evidence of hemodynamic stability.

Blood samples will be collected from the arterial line at the following times: At time of biopsy or tumor resection (day of surgery), 'Time 0' (aligned with dialysate sample collection time above), then 15 and 30 minutes, along with 1, 2, 4, 6, 8, and 24 hours after 'Time 0'.

Blood (3 mL) will be collected in green top tubes containing sodium heparin anticoagulant. The plasma collection tube will be promptly mixed by gently inverting 6-times, then placing it on wet ice, until centrifuged at 1,300 x g for 10 min at 4°C. Samples will be centrifuged for harvesting plasma as soon as possible after collection (within 1 hour). Upon centrifugation, the plasma will be separated from the blood cells using a pipette and transferred into an appropriately labeled polypropylene freezer vial. The samples should be processed to plasma within 30 minutes from centrifugation and the pH adjusted to <4 with the use of 8.5% phosphoric acid (15µL of 8.5% phosphoric acid per 0.5mL of plasma). Plasma will then be stored frozen at -70°C until subsequent batch analysis.

7.2.6 Visit 6 (Post-operative, Day 2)

Control group

The following will be performed for the **research only**:

- Dialysate and blood collection will end at 8 am +/- 4 hours on post-op Day 2.
- Removal of microcatheters.
- Collection of adverse events.

Following completion of dialysate collection, the infusion pump use will cease and the MDC will be removed percutaneously at the bedside by a study team physician. The entry site of the catheter will be closed with a suture or steri-strip as necessary and a clean dressing applied. The patient will be kept in the hospital for observation until discharged by the primary neurosurgical team.

Study drug group

The following will be performed for the **research only**:

- Dialysate and blood sample collection will end at 8 am +/- 4 hours on post-op Day 2.
- Removal of microcatheters.
- Collection of adverse events.

Following completion of dialysate collection, the infusion pump use will cease and the MDC will be removed percutaneously at the bedside by a study team physician. The entry site of the catheter will be closed with a suture or steri-strip as necessary and a clean dressing applied. The patient will be kept in the hospital for observation until discharged by the primary neurosurgical team.

7.2.7 Visit 7 (Post-operative follow-up 14+/-7 days)

Control group

The following will be performed as **routine (standard care)**:

- Neurological exam

The following will be performed for the **research only**:

- Collection of adverse events.

Study drug group

The following will be performed as **routine (standard care)**:

- Neurological exam

The following will be performed for the **research only**:

- Collection of adverse events.

Samples collected during the study for research-only purposes will be sent for analysis as shown below:

Sample	Analysis location
Comprehensive Metabolic Panel (which includes electrolytes, renal function and liver function) at Visits 1, 2, and 5	Clinical laboratory
Tissue from biopsy or surgery	Dr. Connor's laboratory (pharmacodynamics)
	Mass Spectrometry core at Penn State Hershey Cancer Institute (PSHCI) (metabolites and drug concentration)
Plasma at Visits 5 and 6	Mass Spectrometry core at PSHCI (drug concentration)
Dialysate at Visits 5 and 6	Mass Spectrometry core at Penn State Hershey Cancer Institute (PSHCI) (metabolites and drug concentration)

Schedule of events

	Visit 1 (Screening and baseline)	**Visit 2** (Day 3 +/-2 days of azole)***^	**Visit 3** (morning of and during biopsy or surgery)	**Visit 4** (post-op Day 0)	**Visit 5** (post-op Day 1)	**Visit 6** (post-op Day 2)	**Visit 7** (14 days +/- 7 days post-op)
Informed consent process	X						
Demographics[+]	X						
Medical history[+]	X						
Neurological Exam[+]	X			X	X		X
Concomitant medication review[+]	X		X^				
Women only: Pregnancy test[+,] **	X						
12-lead ECG[+]	X	X					
MRI Brain[+]	X*						
Comprehensive Metabolic Panel (which includes electrolytes, renal function and liver function)^	X	X			X		
CBC^	X						
Study drug (posaconazole) dispensed^	X		X#	X#	X#		
Adverse Events (if any)		X	X	X	X	X	X
Microdialysis catheter placement			X				
Intraoperative blood and tissue collection			X				
CT scan of head[+]				X			
Dialysate collection					X	X	
Blood sample collection					X	X	
Microdialysis catheter removal						X	

[+]Performed as routine, standard care (see note about Visit 2) and used for research as well. Any investigations or clinical documentation within 31 days prior to screening are acceptable

* Previously performed MRI Brain +/- Gadolinium contrast scan within 45 days prior visit 1 will be reviewed as baseline MRI

**Only required for women who, in the investigator's opinion, are of childbearing potential

***Only performed for study drug group participants who do not have biopsy or surgery within 3 days. This is a research-only visit and all activities at this visit are for research only.

^Only applicable to those participants in the study drug group.

#As inpatient. Study drug will be dispensed as inpatient on Visit 1 if patient already hospitalized; otherwise it will be outpatient

7.3 Duration of Participation

It will take participants a maximum of 31 days to complete this research study. They will be asked to attend the research site a maximum of 4 separate times.

7.4 Test Article(s) (Study Drug(s) and/or Study Device(s))

7.4.1 Description

Classification:

Posaconazole is a second-generation triazole agent with a potent and broad antifungal activity. Posaconazole is available as concentrated solution to be diluted before intravenous administration, delayed-release tablet, or suspension for oral administration. Posaconazole is designated chemically as 4-[4-[4-[4-[[(3*R*,5*R*)-5- (2,4-difluorophenyl)tetrahydro-5- (1*H*-1,2,4-triazol-1-ylmethyl)-3-furanyl]methoxy]phenyl]-1-piperazinyl]phenyl]-2-[(1*S*,2*S*)-1-ethyl-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one with an empirical formula of C37H42F2N8O4 and a molecular weight of 700.8.

Pharmacokinetics of delayed-release Posaconazole tablets:

When given orally in healthy volunteers, posaconazole delayed-release tablets are absorbed with a median Tmax of 4 to 5 hours. The elimination half-life of posaconazole has been estimated to vary between 15-35 hours [56]. Steady-state plasma concentrations are attained by Day 6 at the 300 mg dose (QD after BID loading dose at Day 1).

The absolute bioavailability of the oral delayed-release tablet is approximately 54% under fasted conditions. The Cmax and AUC of posaconazole following administration of posaconazole delayed-release tablets is increased 16% and 51%, respectively, when given with a high fat meal compared to a fasted state. In order to enhance the oral absorption of posaconazole and optimize plasma concentrations, posaconazole delayed-release tablets should be administered with food.

7.4.2 Treatment Regimen

The timing of study drug administration will be based on the scheduled date and time of biopsy or surgery, which will occur within 7-10 days of Visit 1. The latter parameter is based on the drug half-life, as follows:

- Participants will be taking posaconazole as 300mg PO BID in the first day, followed by 300mg PO once daily thereafter.
- The mean elimination half-life of posaconazole is 26-31 hours (product monograph). Therefore, posaconazole will reach steady state at approximately 130-155 hours following the first dose (5 half-lives). As such, we will work backwards from the scheduled date and time of biopsy or surgery such that participants in the posaconazole arm will receive drug for 168-240 hours. The upper range of the dosing (240 hours) is to minimize potentially unnecessary exposure to drug while accounting for variability in half-life measurements.

In order to maintain steady-state concentrations, study drug will be administered at the following times as well:

1. On the morning prior to planned surgical intervention
2. On the morning of postoperative day 1, at 8:00AM +/- 4 hours *****

*Only if postoperative CT rules out complications and the MDC(s) are correctly positioned.

7.4.3 Method for Assigning Subject to Treatment Groups

Not applicable, as this is an open label, non-randomized Phase 0 feasibility trial.

7.4.4 Subject Compliance Monitoring

Nursing staff will chart administration of study drug into the patient's EMR and study personnel will monitor and document the completion of all study procedures. In the outpatient setting, participants will be provided with a medication diary.

7.4.5 Blinding of the Test Article

Not applicable, as this is an open label, non-randomized Phase 0 feasibility trial.

7.4.6 Receiving, Storage, Dispensing and Return

7.4.6.1 Receipt of Test Article

A study specific supply of FDA-approved posaconazole 100mg tablets will be purchased from a pharmacy wholesaler for use in this study. The manufacturer, lot number, and expiration date of the purchased posaconazole tablets will be maintained in the drug accountability records kept by the IDS pharmacy.

7.4.6.2 Storage

IDS pharmacy will store the posaconazole tablets at controlled room temperature as per the USP guidelines for controlled room temperature.

7.4.6.3 Preparation and Dispensing

Once an enrolled subject's biopsy or surgery date is scheduled, subjects will receive an outpatient supply of the posaconazole tablets to take beginning ~130-155 hours prior to biopsy or surgery. Subjects will take six posaconazole 100mg tablets by mouth, once in the morning and once in the evening, on the first day followed by three 100mg tablets by mouth daily. The last dose from the outpatient supply will be taken on the morning of the biopsy or surgery. Subjects should return any study medication remaining when they arrive the day of biopsy or surgery. Post-biopsy or surgery subjects will receive one additional dose of posaconazole 300mg (three 100mg tablets) from the study supply, in the morning after biopsy or surgery. These doses will also be dispensed from a study specific supply. The medication vial should be return to IDS for accountability.

7.4.6.4 Return or Destruction of the Test Article

Any dispensed but not administered tablets will be noted in the accountability records and subsequently destroyed.

7.4.6.5 Prior and Concomitant Therapy

A concomitant therapy is any medication which is administered between study enrollment and the end of the study drug treatment phase that could achieve a synergistic/antagonistic effect or increase the risk of adverse drug interactions. Situational medications or dose required during the surgical procedures will not be logged. All other therapies will be monitored daily and recorded by study team members during the duration of the treatment phase with the study drug.

The following is a list of contra-indications to the use of posaconazole, as obtained from the product monograph (this list is not all-inclusive):

- Patients who are hypersensitive to this drug or to any ingredient in the formulation or component of the container. For a complete listing, see the Dosage Forms, Composition and Packaging section

of the product monograph. There is no information regarding cross-sensitivity between NOXAFIL® and other azole antifungal agents. Caution should be used when prescribing NOXAFIL® to patients with hypersensitivity to other azoles.

- Co-administration of NOXAFIL® and ergot alkaloids. NOXAFIL® may increase the plasma concentrations of ergot alkaloids, which may lead to ergotism.
- Co-administration of NOXAFIL® and certain medicinal products metabolized through the CYP3A4 system: terfenadine, astemizole, cisapride, pimozide, and quinidine. Although not studied *in vitro* or *in vivo*, co-administration of these CYP3A4 substrates may result in increased plasma concentrations of those medicinal products, leading to potentially serious and/or life-threatening adverse events, such as QT prolongation and rare occurrences of torsade de pointes.
- Co-administration of NOXAFIL® and HMG-CoA reductase inhibitors (statins) that are primarily metabolized through CYP3A4, since increased plasma concentration of these drugs can lead to rhabdomyolysis.
- Co-administration of NOXAFIL® and sirolimus. Concomitant administration of NOXAFIL® with sirolimus increases the sirolimus blood concentrations by approximately 9-fold and can result in sirolimus toxicity.

The following is a full list of drugs that alter the plasma concentration of posaconazole obtained from the product monograph:

- Rifabutin
- Phenytoin
- Efavirenz
- Fosamprenavir
- Glipizide
- Loperamide
- Antacids/H2 receptor antagonists
- Cimetidine
- Esomeprazole
- Metoclopramide

8.0 Subject Numbers and Statistical Plan

8.1 Number of Subjects

Ten (10) subjects will be enrolled in the study.

8.2 Sample size determination

Design

This is an exploratory (pilot) study to assess whether posaconazole can penetrate brain tissue at sufficient enough levels to result in expected downstream biological effects on glycolysis and tumor cell survival. Five evaluable patients will be studied for this trial. The sample size justification is not based on statistical rationale but clinical affordability.

The following metrics will be used to define success and support the decision to proceed with advanced phase trials:

- Detection of ≥ 5uM concentration of either drug in resected tumor in at least 2 of 5 patients in each arm – a value that is within the range needed to achieve efficacy for CNS fungal infections and to achieve 50% glioma tumor cell kill *in vitro* based on our prior published report (ref 58)*;* AND
- Reduction in average lactate and pyruvate concentrations in both resected tumor and dialysate fluid (using MDCs) in the treated cohorts compared to controls; AND
- Steady concentration of lactate and pyruvate over 24 hours of monitoring with MDCs in control participants.

The rationale for the above is based on the notion that any tumor penetration by drug, with concomitant effect on glycolysis, warrants further in-depth analysis as our therapeutic options for these deadly tumors are currently very limited. Intra-tumoral drug concentration on the lower range would still be promising and warrant dose-escalation studies, as the currently selected dosing is based on anti-fungal regimens that are known to be safe. Phase II studies for assessing tumor response would be warranted if substantial tumor penetration together with expected biological effect is observed. A negative trial would nonetheless have value as it would serve as an efficient means of determining whether or not either of ketoconazole or posaconazole hold promise against GBMs, as part of a "fail early" approach to drug-development, while minimizing the human and financial cost investment.

Study Population
Only patients who are eligible and take posaconazole according to protocol specifications (study drug arm only) **and** have undergone successful MDC implantation will be considered evaluable and included in the study analysis. Otherwise, enrolled patients will be replaced. All eligible patients for whom analyzable dialysate samples were obtained for at least 7 of the 9 time points will be considered evaluable for the primary study endpoint. Patients who are not evaluable will be replaced.

8.3 Statistical methods

Primary Objectives
The outcome measure for primary endpoint will be the pharmacokinetics of posaconazole, measured as the area under the curve of the concentration vs time gradient over the first 24 hours (AUC0-24). We will be measuring the concentration of posaconazole (AUC-Drug) in brain interstitium using the MDC. The AUC-Drug will be estimated by the trapezoid rule, based on the number of timepoints available for the particular evaluable patient. Brain posaconazole concentration at steady-state will also be summarized using descriptive statistics. The AUC-Drug will be estimated by standard noncompartmental or compartmental analysis as performed by Phoenix® WinNonlin version 6.3 (Pharsight Corporation, Mountain View, CA, USA).

The PK variables will be tabulated and descriptive statistics calculated. The AUCs will be summarized by mean and standard deviation or median and range if there is large variation from patient to patient. Means and standard deviation or mean and range will be presented for Cmax and AUC.

Secondary Objectives

- Tolerability: Adverse experiences observed over the 7-21 days of protocol follow-up will be characterized in CTCAE v5.0. Frequency and severity of AEs according to body system and severity criteria will be described. In addition, frequency of grade 3 or 4 adverse events will be described separately. Causality will also be noted. Laboratory assessments will also be described according to the NCI CTCAE, V 5.0 criteria, with separate descriptions for grade 3 or 4 laboratory abnormalities. Clinically significant laboratory abnormalities will be described as well.
- Pharmacodynamics: Descriptive statistics, using means/ median, will be used to report drug effect on:
    a) HK2 activity in resected tumor tissue
    b) Concentration of downstream glycolysis metabolites in resected tumor tissue

c) Tumor cell proliferation, cell death, and angiogenesis in resected tumor tissue
d) Concentration vs time profile of lactate and pyruvate, based on MDC studies, in relation to the concentration vs time profile of posaconazole.

** The above values will be compared to levels in the control arm.

Off Study Criteria

Follow-up for this protocol will be considered complete when: (1) intraoperative pathology not consistent with glioma; (2) the patient dies; (3) the patient refuses posaconazole during the preoperative steady-state dosing period; (4) misses more than 3 doses; or (5) completes the 14 +/-7 day follow-up after surgical resection of tumor. If a drug/ procedure-related complication has occurred, the patient will be clinically followed and managed accordingly until resolution.

Patients' disposition will be summarized in the following manner:

- The number and percentage of patients selected, included, completed, withdrawn and lost to follow-up will be summarized using descriptive statistics.
- Major protocol deviations will be summarized.
- The reason for withdrawal (adverse events, major protocol deviation, non-medical reason) will be summarized.

9.0 Data and Safety Monitoring Plan

9.1 Periodic evaluation of data

To monitor safety throughout the course of the study, every effort will be made to remain alert to possible AEs/SAEs. If an AE/SAE occurs, the first concern should be for the safety of the subject. If necessary, appropriate medical intervention will be provided. At the signing of the written consent form, each subject or his/her legally authorized representative and/or main caregiver must be given the names and telephone numbers of study site staff for reporting AEs/SAEs and medical emergencies. In this study, any AE/SAE experienced by the subject between the time of first dose of study treatment and 24 hours after the last dose will be recorded on the electronic case report form, regardless of the severity of the event or its relationship to study treatment. During study treatment and visit after 14 days, the research team will assess the subject for AEs and will record any new AEs/SAEs or updates to previously reported AEs on the electronic case report form.

9.2 Data that are reviewed

Data reviewed are described below.

- Retrieved tumor sample:
  - Drug concentration
  - Hexokinase 2 enzyme activity assay
  - TUNEL staining for assessment of apoptosis
  - Glycolysis metabolites (lactate and pyruvate)
  - Immunohistochemistry – Ki67 staining for assessment of cell proliferation
- Blood and dialysate:
  - Drug concentration
  - Glycolysis metabolites (lactate and pyruvate)
- Clinical safety data:
  - Adverse events, as per NCI CTCAE v 5.0 criteria

9.3 Method of collection of safety information

Safety information will be collected in the research database throughout the study. The safety of the study drug will be evaluated by clinical assessments and laboratory tests (see section 7.2).

9.4 Frequency of data collection

Data collection will occur as follows:

- Laboratory data:
  - Once – Resected tumor, blood samples, and dialysate samples will be analyzed in batch
- Clinical safety data:
  - Monitored at every study visit (please refer to schedule of events)

9.5 Individuals reviewing the data

The following individuals will review the data:

- Dr. Alireza Mansouri (PI)
- Dr. Brad Zacharia (Co-investigator; Co-I)
- Additional research personnel as required

9.6 Frequency of review of cumulative data

Cumulative data will be reviewed once, upon conclusion of the study.

9.7 Statistical tests

For details, see the statistical section under 8.3.

9.8 Suspension of research

This is pilot study, but after periodic evaluation of study result and safety profile, the PI can terminate the research anytime based on outcomes of periodic safety monitoring and benefit/risk outcomes and will inform the IRB if such a decision is being made.

10.0 Risks

Risk of drug

While this drug has never been tested specifically in the HGG population, it is an FDA-approved drug. The potential side effects are:

Common side effects (occurring in at least 1 in 100 patients) are:

- Headache
- Dizziness
- numbness or tingling;
- sleepiness
- feeling or being sick
- loss of appetite
- stomach pain
- diarrhea
- upset stomach
- nausea
- vomiting
- flatulence (excessive gas in the digestive tract)
- dry mouth
- abnormal liver function tests
- rash
- weakness
- tiredness
- a decrease in white blood cells (that can increase the risk of infections)
- fever

- abnormal amounts of salts in the blood

Infrequent

- Liver problems, including liver failure, with symptoms such as:
    - dark colored urine
    - pale stools
    - yellowing of the skin and eyes
    - abdominal pain
    - nausea
    - vomiting

Uncommon

- Heart problems such as very slow, fast or irregular heartbeat

Rare

- Severe allergic reaction with symptoms such as
- severe skin blistering
- peeling rash
- swollen lips, mouth and throat
- difficulty in breathing

Risks of microdialysis
Microdialysis is an invasive surgical procedure with a small risk of bleeding at the site of insertion and discomfort for the patient. Removal of the catheter can result in minor discomfort as well. There is a chance that the catheter may break or get stuck in the brain that would require surgical intervention to remove it.
There is also a small risk of infection (1%), but pre-operative antibiotics are used to reduce the risk. However, this is a small device and it is removed early postoperatively, similar to other surgical drains placed postoperatively. There is also a very small possibility of bleeding along the catheter tip.

Risks of venipuncture
The discomfort associated with removing blood by venipuncture (by a needle from a vein) is a slight pinch or pin prick when the sterile needle enters the skin. The risks include mild discomfort and/or a black and blue mark at the site of puncture. Less common risks include a small blood clot, infection or bleeding at the puncture site, and on rare occasions fainting during the procedure.

Risks of electrocardiogram (ECG):
The patches that the study staff will stick to your chest and other areas of your body to monitor your heart may irritate your skin and cause itching and redness. The study staff might need to shave your body hair so that they can stick the pads to your skin. The shaving may cause some irritation (depending on the tools and soap used); also, a local allergic reaction could occur. When the sticky patches are removed, it might sting for a few seconds. The test itself is painless.

Reproductive risks
The class of drugs, azoles, which the study drug is part of, can cause birth defects. There is only limited information regarding their effect on infants of breastfeeding mothers. For this reason, all women of childbearing potential MUST have a negative pregnancy test.

The study doctor will discuss methods with participants to ensure that they do not become pregnant or father a baby during the study. Women should not breastfeed a baby while taking study treatment and for 30 days after the last dose because the drug used in this study might be present in breast milk and could be harmful to a baby.

Pregnancy statement and use in nursing women
Azoles can be teratogenic.[57] Information regarding adverse effects on infants of breastfeeding mothers taking azoles is limited. All women of childbearing potential MUST have a negative pregnancy test. If the pregnancy test is positive, the patient must not receive any investigational product and must not be enrolled in the study. We will request that female participants wait 30 days after the last dose of drug before considering becoming pregnant or starting breastfeeding.

Definition of childbearing potential: For the purposes of this study, a female of childbearing potential is a sexually mature female who: 1) has not undergone a hysterectomy (the surgical removal of the uterus) or bilateral oophorectomy (the surgical removal of both ovaries) or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time during the preceding 24 consecutive months).

Study participants must be willing to comply with fertility requirements as described below:

- Male participants must agree to use an adequate method of contraception for the duration of the study and for 30 days afterwards.
- Female participants must be either postmenopausal, free from menses ≥ 2 yrs, surgically sterilized, willing to use two adequate barrier methods of contraception to prevent pregnancy, or agree to abstain from heterosexual activity starting with screening and for 30 days afterwards.
- Participants must agree not to donate blood, sperm/ova during study participation and for at least 30 days after stopping treatment.

During the course of the trial, if a participant suspects that they may have conceived a child, they will be instructed to contact the study doctor named at the top of this form immediately. In addition, a missed or late menstrual period during the course of the trial should be also reported to the study doctor

If a male patient is suspected of having fathered a child while on study drug, the pregnant female partner must be notified and counseled regarding the risk to the fetus. In addition, the treating physician must follow the course of the pregnancy, including prenatal and neonatal outcome. Infants should be followed for a minimum of 30 days.

Upon live-birth delivery, the minimum information that should be collected includes date of birth, length of pregnancy, sex of infant, major and minor anomalies identified at birth. Outcomes can be obtained via mailed questionnaires, maternal interviews, medical record abstraction, or a combination of these methods.

Risk of loss of confidentiality
There is a risk of loss of confidentiality if participant information or identity is obtained by someone other than the investigators, but precautions will be taken to prevent this from happening. The confidentiality of participant electronic data created by the participant or by the researchers will be maintained as required by applicable law and to the degree permitted by the technology used. Absolute confidentiality cannot be guaranteed.

11.0 Potential Benefits to Subjects and Others

11.1 Potential Benefits to Subjects

There will be no direct benefit to study participants.

11.2 Potential Benefits to Others

The use of posaconazole as a drug against HGGs has never been formally studied and this is a unique opportunity to study this possible indication. If posaconazole successfully demonstrates effective accumulation in brain and tumor interstitium and result in the expected pharmacodynamics changes, it could be of major importance in improving the outcome of patients with HGGs.

12.0 Sharing Results with Subjects

If the research with the participants identifiable information or samples gives results that do have meaning for their health, the researchers will contact the participants to let them know the findings and discuss the potential implications with them.

13.0 Subject Payment and/or Travel Reimbursements

Participants will receive a $50.00 gift card (via Greenphire) for completing the research-only visit (Visit 2) if it is performed. No compensation will be provided for any other study visits.

14.0 Economic Burden to Subjects

14.1 Costs

There will be no additional costs to the participants for enrolling on the trial.

- The study drug, posaconazole, will be provided at no cost to the study drug group participants while they take part in this study.
- The research-related tests and procedures that will be provided at no cost to the participants include:
  - CMP at Visits 1, 2, and 5, including blood draws (study drug group only_
  - 12-lead ECG at Visit 2 (study drug group)
  - Physical examination at Visit 2 (study drug group)
  - Microdialysis catheter and related reagents, and catheter monitoring at visits 3 through 6 both groups)
  - The removal of the microdialysis catheter will also be at no charge, performed at the bedside (both groups)

14.2 Compensation for research-related injury

It is the policy of the institution to provide neither financial compensation nor free medical treatment for research-related injury. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. Costs for the treatment of research-related injuries will be charged to subjects or their insurance carriers.

15.0 Resources Available

15.1 Facilities and locations

The study will be conducted in the Neurosurgery Clinic, operating room (collection of samples, placement of MDCs), and in post-operative rooms in the hospital at the Penn State Hershey Medical Center.

15.2 Feasibility of recruiting the required number of subjects

The clinical investigators are involved in the care of any patient diagnosed with glioblastoma. Not all will be appropriate study candidates, but the likelihood of identifying and recruiting 10 subjects is high.

15.3 PI Time devoted to conducting the research

The PI and other clinical co-investigators will screen and identify appropriate subjects as part of their routine clinical activity of caring for patients. The clinical investigators are supported by a team of clinical research staff who will assist in the completion of the research procedures. All clinical members of the research team are experienced clinicians and researchers, who have successfully balanced their clinical and research responsibilities.

15.4 Availability of medical or psychological resources

All resources and facilities of the HMC are available to subjects on study, as part of their routine standard of care. No additional resources are anticipated to be needed specifically due to participation in this study.

15.5 Process for informing Study Team

Members of the research team will be required to undergo protocol training and to sign a delegation of duties log prior to participation in the study. During the study, the PI and/or study coordinator will communicate updates to all team members as they become aware of any changes or updates.

16.0 Other Approvals

16.1 Other Approvals from External Entities

Will need approval from grant funders.

16.2 Internal PSU Committee Approvals

Check all that apply:

☒ Anatomic Pathology – **Penn State** Health **only** – Research involves the collection of tissues or use of pathologic specimens. Upload a copy of "HRP-902 - Human Tissue For Research Form" in CATS IRB.

☐ Animal Care and Use – **All campuses** – Human research involves animals and humans or the use of human tissues in animals

☒ Biosafety – **All campuses** – Research involves biohazardous materials (human biological specimens in a PSU research lab, biological toxins, carcinogens, infectious agents, recombinant viruses or DNA or gene therapy).

☒ Clinical Laboratories – **Penn State Health only** – Collection, processing and/or storage of extra tubes of body fluid specimens for research purposes by the Clinical Laboratories; and/or use of body fluids that had been collected for clinical purposes but are no longer needed for clinical use. Upload a copy of "HRP-901 - Human Body Fluids for Research Form" in CATS IRB.

☐ Clinical Research Center (CRC) Advisory Committee – **All campuses** – Research involves the use of CRC services in any way.

☐ Conflict of Interest Review – **All campuses** – Research has one or more of study team members indicated as having a financial interest.

☒ Radiation Safety – **Penn State Health only** – Research involves research-related radiation procedures. All research involving radiation procedures (standard of care and/or research-related) must upload a copy of "HRP-903 - Radiation Review Form" in CATS IRB.

☐ IND/IDE Audit – **All campuses** – Research in which the PSU researcher holds the IND or IDE or intends to hold the IND or IDE.

☒ Scientific Review – **Penn State Health only** – All investigator-written research studies requiring review by the convened IRB must provide documentation of scientific review with the IRB submission. The scientific review requirement may be fulfilled by one of the following: (1) external peer-review process; (2) department/institute scientific review committee; or (3) scientific review by the Clinical Research Center Advisory committee. NOTE: Review by the Penn State Health Cancer

Institute (PSCI) Protocol Review Committee or the PSCI Disease Team is required if the study involves cancer prevention studies or cancer patients, records and/or tissues. For more information about this requirement see the IRB website.

17.0 Multi-Site Study

17.1 Other sites
Not applicable

17.2 Communication Plans
Not applicable

17.3 Data Submission and Security Plan
Not applicable

17.4 Subject Enrollment
Not applicable

17.5 Reporting of Adverse Events and New Information
Not applicable

17.6 Audit and Monitoring Plans
Not applicable

18.0 Adverse Event Reporting

18.1 Adverse Event Definitions

For drug studies, incorporate the following definitions into the below responses, as written:	
Adverse event	Any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related
Adverse reaction	Any adverse event caused by a drug
Suspected adverse reaction	Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction". • *Reasonable possibility*. For the purpose of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.
Serious adverse event or Serious suspected adverse reaction	Serious adverse event or Serious suspected adverse reaction: An adverse event or suspected adverse reaction that in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
Life-threatening adverse event or life-threatening suspected adverse reaction	An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator (i.e., the study site principal investigator) or Sponsor, its occurrence places the patient or research subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that had it occurred in a more severe form, might have caused death.
Unexpected adverse event or Unexpected	An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure, general investigational plan, clinical

suspected adverse reaction.	protocol, or elsewhere in the current IND application; or is not listed at the specificity or severity that has been previously observed and/or specified.

For device studies, incorporate the following definitions into the below responses, as written:	
Unanticipated adverse device effect	Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or IDE application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

18.2 Recording of Adverse Events

Subjects will be encouraged to spontaneously report any AE. Study personnel will ask open-ended questions to obtain information about AEs at every visit. Date and time of onset and resolution (if applicable) of the AE will be documented. All AEs occurring after the initiation of the study treatment (treatment emergent adverse events) will be reported, including events present at baseline that worsened during the study.

Safety will be assessed by the investigator in the form of evaluation of adverse events and clinical laboratory results and derived as data sets and frequency methods, recorded for this study. AEs by severity and causality as described below:

- Laboratory evaluations including liver function tests. Laboratory values within 1.5x the institutional upper limit of normal are considered acceptable and not as AEs

To monitor any risk of increased surgical complications associated with posaconazole, the treating surgeons will make a Note-to-File recording any unexpected events that occurred during surgery.

Patients unable to tolerate posaconazole due to AE grade 3-4 (hepatic, gastroenterological, and/or renal toxicity) will have a dose reduction. Posaconazole will be reduced to 33% of schedule dose (200 mg per dose, see table below). If the AE does not resolve within 24 hours, it will be discontinued. However, tumor tissue will still be collected and analyzed.

Posaconazole **Dose Modification for Adverse Reactions**

Dose Level	Posaconazole dose
Starting Dose	300 mg once daily
Dose Reduction (33%)	200 mg once daily

Pregnancy statement

During the course of the trial, all patients of childbearing potential should be instructed to contact the treating physician immediately if they suspect they might have conceived a child. In addition, a missed or late menstrual period should be reported to the treating physician. If a female patient or the treating physician suspects that the female patient may be pregnant prior to administration of study drugs, the study drugs must be withheld until the results of a pregnancy test are available. If pregnancy is confirmed the patient must not receive study medications and must be withdrawn from the study. Throughout the entire pregnancy, additional contact should be made with the patient, and in some cases with the healthcare provider, to identify spontaneous abortions and elective terminations, as well as any medical reasons for elective termination. In addition, the study investigator should include perinatal and neonatal outcome. Infants should be followed for a minimum of 30 days.

If a male patient is suspected of having fathered a child while on study drugs, the pregnant female partner must be notified and counseled regarding the risk to the fetus. In addition, the treating physician must follow the course of the pregnancy, including prenatal and neonatal outcome. Infants should be followed for a minimum of 30 days.

Upon live-birth delivery, the minimum information that should be collected includes date of birth, length of pregnancy, sex of infant, major and minor anomalies identified at birth. Outcomes can be obtained via mailed questionnaires, maternal interviews, medical record abstraction, or a combination of these methods.

18.3 Causality and Severity Assessments

The investigator will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the study drug(s) or device(s); and 3) if the adverse event meets the criteria for a serious adverse event.

If the investigator's final determination of causality is "unknown and of questionable relationship to the study drug(s) or device(s)", the adverse event will be classified as associated with the use of the study drug(s) or device(s) for reporting purposes. If the investigator's final determination of causality is "unknown but not related to the study drug(s) or device(s)", this determination and the rationale for the determination will be documented in the respective subject's case history.

18.4 Reporting of Adverse Reactions and Unanticipated Problems to the FDA

Not applicable

18.4.1 Written IND/IDE Safety Reports

Not applicable

18.4.2 Telephoned IND Safety Reports – Fatal or Life-threatening Suspected Adverse Reactions

Not applicable

18.5 Reporting Adverse Reactions and Unanticipated Problems to the Responsible IRB

In accordance with applicable policies of The Pennsylvania State University Institutional Review Board (IRB), the investigator will report, to the IRB, any observed or reported harm (adverse event) experienced by a subject or other individual, which in the opinion of the investigator is determined to be (1) unexpected; and (2) probably related to the research procedures. Harms (adverse events) will be submitted to the IRB in accordance with the IRB policies and procedures.

18.6 Unblinding Procedures

Not applicable

18.7 Stopping Rules

Trial accrual will be prematurely stopped if two or more unacceptable toxicities occur. Unacceptable toxicity is defined as mortality or grade 4 toxicity directly attributed to drug.

19.0 Study Monitoring, Auditing and Inspecting

19.1 Study Monitoring Plan

19.1.1 Quality Assurance and Quality Control

The study will be monitored by Clinical Trial Monitoring Team from the Department of Public Health Sciences at Penn State Hershey College of Medicine.

Staff within the Department of Public Health Sciences will be monitoring the study for protocol compliance, data quality, and regulatory compliance. This will include reviewing the informed

consent process and completed forms, verifying the presence of essential documents in the study regulatory binder, completing source document verification for data entered into REDCap, ensuring the study is implemented as planned, reviewing adverse events and the reporting of serious adverse events, and ensuring that all data quality rules have been executed and resolved and all data queries are resolved and closed.

The monitor will create a detailed report following each scheduled monitoring session to forward to the PI and will verify that proposed action items are addressed and completed. The monitoring will occur at regular intervals as specified in the monitoring plan developed by Public Health Sciences and PSU Sponsor Investigator.

19.1.2 Safety Monitoring

Data and safety will be monitored by the Cancer Institute Data Safety Monitoring Committee (CI DSMC).

The Principal Investigator will confirm that all adverse events (AE) are correctly entered into the AE case report forms by the coordinator; be available to answer any questions that the coordinators may have concerning AEs; and will notify the IRB, FDA, and/or DSMB of all applicable AEs as appropriate. All assessments of AEs will be made by a licensed medical professional who is an investigator on the research.

The Research Coordinator will complete the appropriate report form and logs; assist the PI to prepare reports and notify the IRB, and CI DSMC of all Unanticipated Problems/SAE's.

The Monitor will confirm that the AEs are correctly entered into the case report forms. The Monitor will also confirm that the adverse events are consistent with the source documents and are reported to the appropriate regulatory bodies as required.

20.0 Future Undetermined Research: Data and Specimen Banking

20.1 Data and/or specimens being stored
Not applicable

20.2 Location of storage
Not applicable

20.3 Duration of storage
Not applicable

20.4 Access to data and/or specimens
Not applicable

20.5 Procedures to release data or specimens
Not applicable

20.6 Process for returning results
Not applicable

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22.0 Confidentiality, Privacy and Data Management

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