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Official Study Title: **A Phase 2, Investigator Initiated Study to Determine the Safety and Efficacy of TH-302 in Combination with Bevacizumab for Glioblastoma Following Bevacizumab Failure (CTRC# 12-0105)**

NCT number: NCT02342379

IRB Approval Date: 04/04/17

Unique Protocol ID: CTRC 12-0105

UT MEDICINE
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HEALTH SCIENCE CENTER SAN ANTONIO

INSTITUTE FOR DRUG DEVELOPMENT

A Phase 2, Investigator Initiated Study to Determine the Safety and Efficacy of TH-302 in Combination with Bevacizumab for Glioblastoma Following Bevacizumab Failure

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IND# **116977**

CTRC# **12-0105**

Protocol Version 5.0, dated March 31, 2017
Protocol Version 4.0, dated November 19, 2015
Protocol Version 3.0, dated April 22, 2015
Version 2.0, dated September 15, 2014
Version 1.5, dated March 7, 2014

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SYNOPSIS

Study Title:	A Phase 2, Investigator Initiated Study to Determine the Safety and Efficacy of TH-302 in Combination with Bevacizumab for Glioblastoma Following Bevacizumab Failure
Investigators:	Andrew J. Brenner MD, PhD Eudocia Quant Lee, MD *See 1572 for other investigators*
Indication	Recurrent High Grade Glioma progressing on Conventional Chemoradiation with Temozolomide as well as Salvage Bevacizumab
Primary Objectives:	• To assess the safety of TH-302 in combination with bevacizumab for patients with high grade glioma • To determine the progression-free survival (PFS) for patients treated with combination bevacizumab and TH-302 following recurrence on single agent bevacizumab
Secondary Objectives:	• To evaluate overall survival of patients for patients treated with combination bevacizumab and TH-302 following recurrence on single agent bevacizumab • To assess preservation of Quality of Life (QOL) using the MD Anderson Symptom Inventory for Brain Tumors (MDASI-BT).
Exploratory Objectives:	• To explore the extent of tumor hypoxia and cerebral blood flow *in vivo* via ^{18}F-FMISO Positron Emission Tomography with correlation to patient response and survival • To explore the role of emerging biomarkers of hypoxia in predicting response to TH-302 and overall survival
Study Design:	Multi-center, single arm, two-stage, non-blinded, prospective study of combination therapy bevacizumab at 10mg/kg and TH-302 at 670mg/m^2 every 2 weeks (6 week cycle) until disease progression. TH-302 will be administered by IV infusion over 30-60 minutes on Days 1, 15, and 29 of a 42-day cycle.
Duration:	Subjects will be allowed to continue treatment until they have evidence of significant treatment-related toxicity or progressive disease.
Planned Total Sample Size:	33 Patients.
Inclusion Criteria	1. At least 18 years of age 2. Ability to understand the purposes and risks of the study and has signed a written informed consent form approved by the investigator's IRB/Ethics Committee 3. Histologically confirmed glioblastoma 4. Progression following both standard combined modality treatment with radiation and temozolomide chemotherapy, as well as bevacizumab 5. Recovered from toxicities of prior therapy to grade 0 or 1 6. ECOG performance status ≤ 2

	7. Life expectancy of at least 3 months 8. Acceptable liver function: a. Bilirubin ≤ 1.5 times upper limit of normal b. AST (SGOT) and ALT (SGPT) ≤ 3.0 times upper limit of normal (ULN); 9. Acceptable renal function: a. Serum creatinine ≤ULN 10. Acceptable hematologic status (without hematologic support): a. ANC ≥1500 cells/uL b. Platelet count ≥100,000/uL c. Hemoglobin ≥9.0 g/dL 11. All women of childbearing potential must have a negative serum pregnancy test and male and female subjects must agree to use effective means of contraception (surgical sterilization or the use or barrier contraception with either a condom or diaphragm in conjunction with spermicidal gel or an IUD) with their partner from entry into the study through 6 months after the last dose
Exclusion Criteria:	1. The subject is receiving warfarin (or other coumarin derivatives) and is unable to switch to low molecular weight heparin (LMWH) before the first dose of study drug. 2. The subject has evidence of acute intracranial or intratumoral hemorrhage either by MRI or computerized tomography (CT) scan. Subjects with resolving hemorrhage, punctate hemorrhage, or hemosiderin are eligible. 3. The subject is unable to undergo MRI scan (eg, has pacemaker). 4. The subject has received enzyme-inducing anti-epileptic agents within 14 days of study drug (eg, carbamazepine, phenytoin, phenobarbital, primidone). 5. The subject has not recovered to National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03 Grade ≤ 1 from AEs (except alopecia, anemia and lymphopenia) due to surgery, antineoplastic agents, investigational drugs, or other medications that were administered prior to study drug. 6. The subject has evidence of wound dehiscence 7. Severe chronic obstructive or other pulmonary disease with hypoxemia (requires supplementary oxygen, symptoms due to hypoxemia or oxygen saturation <90% by pulse oximetry after a 2 minute walk) or in the opinion of the investigator any physiological state likely to cause normal tissue hypoxia 8. The subject is pregnant or breast-feeding. 9. The subject has serious intercurrent illness, such as: a. hypertension (two or more blood pressure [BP] readings performed at screening of > 150 mmHg systolic or > 100 mmHg diastolic) despite optimal treatment b. non-healing wound, ulcer, or bone fracture c. significant cardiac arrhythmias

	d. untreated hypothyroidism e. uncontrolled active infection f. symptomatic congestive heart failure or unstable angina pectoris within 3 months prior study drug g. myocardial infarction, stroke, transient ischemic attack within 6 months h. gastrointestinal perforation, abdominal fistula, intra-abdominal abscess within 1 year i. history or clinical evidence of pancreatitis within 2 years 10. The subject has inherited bleeding diathesis or coagulopathy with the risk of bleeding. 11. The subject has received any of the following prior anticancer therapy: a. Non-standard radiation therapy such as brachytherapy, systemic radioisotope therapy, or intra-operative radiotherapy (IORT). Note: stereotactic radiosurgery (SRS) is allowed b. Non-bevacizumab systemic therapy (including investigational agents and small-molecule kinase inhibitors) or non-cytotoxic hormonal therapy (eg, tamoxifen) within 7 days or 5 half-lives, whichever is shorter, prior first dose of study drug c. Biologic agents (antibodies, immune modulators, vaccines, cytokines) within 21 days prior to first dose of study drug, with the exception of bevacizumab which can be 14 days or maintain the subjects current bevacizumab dosing schedule d. Nitrosoureas or mitomycin C within 42 days, or metronomic/protracted low-dose chemotherapy within 14 days, or other cytotoxic chemotherapy within 28 days, prior to first dose of study drug e. Prior treatment with carmustine wafers f. Prior treatment with TH-302
Assessments of: • **Efficacy** • **Safety**	Progression-free survival at 4 months Safety endpoints • Incidence and severity of adverse events • Changes in lab parameters, vital signs and weight
Procedures (Summary)	Subjects will receive study drug TH-302 and bevacizumab and will be assessed for adverse events at all visits. Adverse events will be reported for all TH-302 related events occurring after the start of treatment until 30 days after study drug is discontinued or subsequent cancer therapy is initiated. Tumor assessments will be performed after every cycle during treatment.

1. INTRODUCTION

1.1 SCIENTIFIC BACKGROUND

1.1.1 Glioblastoma

Primary CNS tumors account for 1.4% of all cancers and 2.4% of all cancer-related deaths. In 2010, an estimated 22,020 new cases of primary malignant brain tumors will be diagnosed and 13,140 patients will die from these tumors. Approximately 50% of all primary brain tumors originate from astrocytes, one of the subtypes of the nonneuronal supportive glial cells of the brain. The second histological type of primary brain tumors are derived from oligodendrocytes and called oligodendrogliomas. Primary brain tumors that develop from astrocytes are called astrocytomas. The World Health Organization (WHO) classifies astrocytomas into four distinct grades referred to as Grades I, II, III and IV on the basis of how quickly the cells grow and spread and how the cells appear under a microscope. Essentially all grade II and III astrocytomas will progress to grade IV during the disease course. Glioblastoma (GBM, Grade IV astrocytoma) is the most common and most aggressive of the primary malignant brain tumors in adults. Annually there are approximately 13,000 cases of GBM diagnosed, with historical 1 year and 5 year survival rates of 29.3% and 3.3%[1]. The poor survival is attributable partly to the nature of the tumor. The infiltrative nature of GBM results in difficulty eliminating microscopic disease despite macroscopic gross-total resection, with 90% of patients having recurrence at the original tumor location[2]. The location of the tumor also makes drug delivery difficult with only small or lipophilic molecules able to cross the blood brain barrier to reach the tumor. Of those agents that are able to reach the tumor, GBMs have shown to be resistant to most cytotoxic agents and to quickly develop resistance when initially sensitive. The most significant advance in treatment for GBM over the last several years has come from concomitant chemoradiotherapy with temozolamide which can result in increased median survival of 14.6 months with radiotherapy plus temozolomide compared to 12.1 months with radiotherapy alone[3]. However, this still leaves much to be desired with an improvement in median survival of only 2.6 months over radiotherapy alone. In the recurrent and progressive setting there is no clear standard of care. Current studies have shown that some benefit can be seen with the topisomerase-1 inhibitor Irinotecan combined with the angiogenesis inhibitor Avastin. This combination can achieve median progression free survivals of 22- 23 weeks, and median overall survivals of 40 to 54 weeks[4, 5].

It is well accepted that brain tumors rely on vascularization for survival and continued growth. While normal brain vasculature is a highly organized structure of endothelial cells, pericytes, and astrocytes forming a tight barrier which restricts access to the intracerebral system, GBMs display markedly disorganized vascular structures with conspicuous endothelial cell proliferation, pericyte and basement membrane abnormalities resulting in permeability with heterogenous leakiness and abnormal blood flow[6]. This aberrant process of endothelial proliferation and loss of the blood brain barrier is related to the expression of vascular endothelial growth factor (VEGF) by the tumor cells as well as the cells in the surrounding microenvironment in response to hypoxia and acidosis. This leakiness results in an increased interstitial pressure and an impediment to drug delivery. By blocking VEGF signaling either through monoclonal antibodies to VEGF-A or inhibition of the VEGF receptors, it has been shown that prompt reduction in interstitial

pressures can be achieved with presumed better drug delivery to the tumor cells. While the role of VEGF signaling in angiogenesis has been extensively described, another role for VEGF has been proposed with carcinoma progression selecting for cells that depend on VEGF as a survival and migration factor[7]. This would explain the high frequency of VEGF receptor expression in GBMs as well as other malignancies [8]. Targeting of VEGF has proven to be clinically relevant in recurrent glioblastoma as further discussed below. Current studies are addressing the impact of angiogenic inhibition upfront in newly diagnosed glioblastoma.

1.1.2 TH-302

TH-302 is a nitroimidazole prodrug of the cytotoxin, bromo-isophosphoramide mustard (Br-IPM). When exposed to hypoxic conditions, TH-302 is reduced at the nitroimadazole site of the prodrug by intracellular reductases leading to the release of the alkylating agent Br-IPM. Br-IPM can then act as a DNA crosslinking agent. It may also diffuse to adjacent cells in normoxic regions and thus act as a cytotoxic agent outside of the hypoxic activation zone. *In vitro* cytotoxicity and clonogenic assays indicate that TH-302 has little activity under normoxic conditions but is highly cytotoxic under hypoxic conditions.

TH-302 does not serve as a substrate for the clinically relevant efflux pumps tested. These include MDR1 (ABCB1), MRP1 (ABCC1) and BCRP (ABCG2).

Few normal cells ever reach severe hypoxia. In contrast, tumors often consist of large areas of highly hypoxic cells that are known to be resistant to chemotherapy and radiation treatment [9, 10]. In areas of normoxia, TH-302 remains intact as a prodrug and toxicity is minimized. Thus, TH-302 has been designed to be selectively activated in these highly hypoxic tumor regions and this makes it an attractive candidate for clinical development. In addition, preclinical data suggest that after activation, the active moiety may diffuse to areas outside the hypoxic region, demonstrating a "bystander" effect and possibly exhibiting additional antitumor activity.

1.1.3 Nonclinical Studies with TH-302

1.1.3.1 Mechanism of Action

TH-302 has been tested under various levels of oxygen in proliferation and clonogenic assays using a number of human cancer cell lines. Initial studies were conducted on H460 lung cancer and HT-29 colon cancer cell lines. Cells were exposed to varying oxygen conditions and TH-302 for two hours and then followed for 3 days. The cytotoxic effect of TH-302 in a 3 day proliferation assay with H460 cells was both dose and oxygen dependent with a greater than 10-fold increase in cell kill (based on IC_{50}) under 0.6% oxygen levels and a 500-fold increase in the absence of oxygen as compared with air (20% oxygen). In normoxic conditions the drug levels necessary to achieve cytotoxic effects were well above those expected to be achieved *in vivo.* A similar oxygen dependent shift of the effect of TH-302 on proliferation was observed with HT-29 cells. Clonogenic assays also provided similar results. We then tested the hypoxia-selective cytotoxicity profile of TH-302 across a wide range of different human cancer cell lines (>30). Every line examined exhibited a greatly enhanced cytotoxicity when the TH-

302 exposure under hypoxic conditions (2h) is compared to the potency observed with a 2h exposure under normoxia. Of particular note was the finding of a wide range of potencies that spanned more than two orders of magnitude among the different individual cell lines examined (0.1 μM – 88μM).

1.1.3.2 Human Tumor Xenograft Studies

TH-302 antitumor activity as monotherapy was dose-dependent and correlated with total drug exposure. Correlation was found between antitumor activity and tumor hypoxic fraction across the twelve xenograft models profiled. Tumor-bearing animals breathing 95% O_2 during dosing periods exhibited attenuated TH-302 efficacy, with while those breathing 10% O_2 exhibited enhanced TH-302 efficacy, both compared to normoxia (21% O_2) breathing. TH-302 treatment results in a reduction in the volume of the hypoxic fraction 48 hr after dosing and a corresponding increase in the necrotic fraction. TH-302 induced DNA damage as measured by γH2AX was initially only present in the hypoxic regions and then radiated to the entire tumor in a time-dependent manner.

To identify the optimal dosing regimen for *in vivo* anti-tumor efficacy of TH-302 in the H460 model, we performed a study employing different dosing regimens of TH-302 in combination with docetaxel. Tumor-bearing mice were treated ip with TH-302 according to three different regimens: 50mg/kg QDx5/wk x2wk; 100mg/kg Q3Dx5; and 150mg/kg Q7Dx2. TH-302 monotherapy, tumor growth was inhibited 89%, 89% and 94% and the TGD_{500} compared to vehicle control were 18, 16 and 16 days, with these three regimens respectively. TH-302 monotherapy did not show significant body weight loss.

The antitumor activity of TH-302 was investigated in combination with seven commonly used chemotherapeutics (docetaxel, cisplatin, pemetrexed, irinotecan, doxorubicin, gemcitabine and temozolomide) in nine human tumor xenograft models, including H460 and Calu6 non-small cell lung cancer (NSCLC), HT29 colon cancer, PC3 prostate cancer, HT1080 fibrosarcoma, A375 and Stew2 melanoma, and Hs766t, MiaPaCa-2, BxPC-3, and SU.86.86 pancreatic cancer. Different TH-302 dosing sequences and regimens in combination with the conventional chemotherapeutics were explored. The antitumor activity of docetaxel, cisplatin, pemetrexed, irinotecan, doxorubicin, gemcitabine and temozolomide was increased when combined with TH-302 in seven out of nine models tested. The two models that did not yield a statistically significant antitumor effect from the combination therapy were both tumor models exhibiting very high vascularity in their tumors, and correspondingly very low hypoxic fractions.

TH-302 has been tested in two orthotopic models of cancer. Each of these models provided evidence of the superior activity of TH-302 in combination with standard chemotherapeutics. In the first orthotopic model, TH-302 was studied in nude mice with red-fluorescent protein (RFP) expressing MIA PaCa-2 tumors that were surgically implanted on the surface of the pancreas under anesthesia[11]. After three days the mice were randomized into groups of 8 and were treated with vehicle; TH-302 at 30 mg/kg, IP (intraperitoneal), once a day for 5 days repeated for a total of 11 doses over 15 days; gemcitabine IV at 200 mg/kg once a week for three doses over 15 days; or the combination of the two drugs. Monotherapy with TH-302 provided minor reductions in tumor growth

whereas gemcitabine alone significantly inhibited tumor growth and increased survival significantly. The combination of TH-302 and gemcitabine provided greatly enhanced reduction in tumor volume. In addition, this combination provided greater survival than gemcitabine alone. In the second orthotopic model, TH-302 was studied in nude mice with green-fluorescent protein (GFP) expressing PC-3 prostate tumors that were surgically implanted on the surface of the prostate under anesthesia [12]. Three days after the orthotopic transplantation, the mice were randomized into groups of 8 and were treated with vehicle; TH-302 at 30 or 50 mg/kg, IP, once a day for 5 days, repeated for two weeks; paclitaxel IV at 12 mg/kg every three days for 4 weeks; or the combination of the two drugs at both doses of TH-302. Paclitaxel significantly reduced mean tumor volume and significantly improved survival. There was a delay in tumor growth in the group treated only with TH-302 at either 30 or 50 mg/kg. However, all tumors did begin to grow later in the study, particularly after the end of treatment. The greatest reductions in tumor volume occurred in groups treated with both agents regardless of TH-302 dose. In these groups 5/8 mice (30 mg/kg) and 3/8 mice (50 mg/kg) had complete responses in the prostate tumor. Open body images collected at the end of the study confirmed the absence of fluorescent signal for four of these mice in the 30 mg/kg group and all three mice in the 50 mg/kg group.

TH-302 has been tested in two metastatic mouse models. Each of these models provided evidence of the superior activity of TH-302 in combination with standard chemotherapeutics. A PC-3/luc intracardiac injection model was performed to evaluate the efficacy of TH-302 and TH-302 in combination with docetaxel in a model of metastases in the bone and soft tissue. Treatments consisted of vehicle, docetaxel alone (20 mg/kg once a week for 3 weeks, IV), TH-302 alone (50 mg/kg daily IP, 5 days a week for 3 weeks) or the combination of the two drugs. In addition to metastases in the mandible, fore or hind limbs, most mice also developed soft tissue metastases primarily in the liver and lungs. Mice treated with TH-302 alone had a 95% reduction in bone metastases and a 96% reduction in soft tissue metastases at Day 42. Mice treated with docetaxel alone had a significant reduction in both bone and soft tissue metastases with 3 of 10 (30%) mice showing a complete response initiating on Day 21 or Day 28 and continuing through study termination on Day 56. Mice treated with the combination of TH-302 and docetaxel demonstrated the greatest decrease in both bone and soft tissue metastases. Eight of ten (80%) of mice had a complete response (bone and soft tissue metastases). Toxicities of the combination regimen were similar to that of the docetaxel alone group with one animal in each group having a treatment related death. All eight mice in the TH-302 and docetaxel combination group who survived to the end of the study had complete responses of the bone metastases and soft tissue metastases. A Nci-H460 pleural space implantation model was performed to evaluate the efficacy of TH-302 alone and in combination with docetaxel[13]. Treatments started seven days after implantation and consisted of vehicle, docetaxel alone (10 mg/kg once a week for 2 weeks, IV), TH-302 alone (50 mg/kg IP daily, 5 days a week for 2 weeks) or the combination of the two drugs. Median survival was 24 days for vehicle compared to 42.5 days with TH-302, 35 days for docetaxel alone and 56 days with TH-302 in combination with docetaxel.

TH-302 has also been tested in ectopic tumor xenograft models in combination with clinically approved VEGFR kinase antiangiogenesis agents (sunitinib and sorafenib). Several published studies have demonstrated an increase in tumor hypoxia after

administration of anti-angiogenesis agents that target VEGF signaling (e.g. sunitinib, sorafenib, bevacizumab). To characterize sunitinib-induced effects on tumor vasculature and tumor hypoxia, 786-O (RCC) and H460 (NSCLC) human ectopic tumor xenografts were treated with sunitinib (20 or 40 mg/kg) daily for 5 days and then 72 hours later animals were injected with pimonidazole to label hypoxic cells and Hoechst 33342 to label vascular perfusion. The NSCLC model (H460) exhibits a baseline hypoxic fraction of 7%. Sunitinib induced a dose-dependent increase in tumor hypoxia volume (24 ± 3.2% with 40 mg/kg vs. 7.3 ± 3.8% with Vehicle, $p<0.05$) and a corresponding decrease in tumor microvasculature. The 786-O RCC model is a well-vascularized tumor as characterized by CD31 and Hoechst staining with a relatively small baseline hypoxic compartment (<5% volume) in the xenograft tumors. Sunitinib also induced a dose-dependent increase in tumor hypoxia volume (17.2 ± 7.1% with 40 mg/kg vs. 1.4 ± 0.9% with Vehicle, $p<0.05$) and a corresponding decrease in functional vasculature in the RCC model. To test the combination of TH-302 with sunitinib, H460 NSCLC tumor-bearing animals (~150mm3 at start of dosing) were randomized into groups of 10 and treated with sunitinib at 20, 40, or 80 mg/kg p.o. daily for 3 weeks (QDx21). After one week of sunitinib monotherapy animals began combination therapy with the addition of TH-302 at 50 mg/kg i.p. daily for 5 days on and 2 days off (QDx5) for 2 weeks. Sunitinib was administered 4 hours before TH-302 on days when both agents were given. Control arms included all 3 doses of sunitinib monotherapy, TH-302 monotherapy, and vehicles-only groups. Sunitinib exhibited dose-dependent antitumor efficacy (by TGI, TGD, and conditional survival), as did TH-302 monotherapy. All three combination therapy treatment groups exhibited superior efficacy compared to the corresponding monotherapy groups. A similarly designed experiment was performed in the RCC (786-O) model with sunitinib and the H460 NSCLC and A375 melanoma models with sorafenib, and similar results were obtained of TH-302 addition potentiating the antiangiogenic antitumor effects. The results support the hypothesis that the hypoxia-activated prodrug TH-302 may be an effective addition to antiangiogenic-containing chemotherapy regimens.

1.1.3.2.1 Preclinical Pharmacokinetic Studies

The intravenous pharmacokinetics of TH-302 have been studied in mice, rats, dogs, and monkeys. In all species, the drug was cleared rapidly from the circulation with mean effective half-lives of 10-50 minutes. TH-302 was stable when incubated with hepatic microsomes from mice, rats, dogs, monkeys and humans. TH-302 was relatively stable in rat, dog and human hepatocytes but less stable in mouse and monkey hepatocytes. TH-302 caused no apparent inhibition of CYP1A2, CYP2C19, CYP2D6 and CYP3A4, but exhibited weak drug-drug interaction potential towards CYP2C9. TH-302 is extensively metabolized following IV administration and is renally and fecally excreted in rat and dog. A tissue distribution study in rats showed that TH-302 is rapidly distributed to tissues following IV administration with the highest concentrations of TH-302-related radioactivity present in the kidney and small intestinal content; however, low but quantifiable levels of radioactivity were observed in the brain and spinal cord. Protein binding was low (<55%) in mouse, rat, dog, monkey and human plasma and independent of plasma concentration.

Following IV administration of [^{14}C]-TH-302 to male rats, radioactive material was distributed rapidly into the tissues evaluated and then eliminated rapidly over the 24 hours after dosing. The highest concentrations of radioactive material within the 24 hours after

dosing were found in the kidney and the GI tract. The presence of substantial amounts of radioactive material in the GI tract suggests the presence of either significant biliary excretion and/or secretion into the gut of TH-302–derived radioactivity. The high concentrations of radioactive material in the urinary bladder contents and the kidney indicate that the kidney also contributes to the elimination of TH-302-derived radioactivity. Low concentrations of radioactive material were found in the brain and spinal cord, indicating low passage of radioactive material across the blood-brain barrier and subsequent protracted elimination from CNS tissues.

1.1.3.2.2 Acute and Subacute Toxicity Studies

Initial single-dose toxicity studies of TH-302 were conducted in rats and dogs. The infusion period was limited to 30 minutes in order to achieve a rapid increase to relatively high drug levels. At the highest dose levels of 100 and 200 mg/kg in rats and 32 mg/kg in dogs, TH-302 was lethal. Organs affected in both species were bone marrow, kidney, thymus and intestine. Species specific toxicity at the highest dose was seen in rat adipose tissue and spleen. Minimal reversible toxicity was observed in rats at 50 mg/kg and in dogs at 8 mg/kg. Based on these results, doses of 12.5, 25 and 50 mg/kg of TH-302 were administered in a GLP multiple-dose toxicity study in rats. Each rat received a 30 minute infusion once a week for three weeks. The primary toxicities were hematologic in nature and were reversible. These effects were pronounced only at the highest dose (50 mg/kg) at which histopathology revealed effects on the bone marrow and the tongue with minor effects on the kidney and the uterus. The minimally toxic multiple-dose in rats was 25 mg/kg. In the repeat dose GLP study with dogs receiving 4, 8, or 16 mg/kg of TH-302, leukopenia and neutropenia were evident, particularly at the high dose level where hypocellularity was also reported in the bone marrow. These changes were reversible. There was no sign of vein irritation in either species tested and exposure of human blood to high levels of TH-302 did not reveal signs of hemolysis. The minimally toxic multiple-dose level in dogs was 8 mg/kg.

1.1.4 Clinical Studies with TH-302

Study TH-CR 401 is a Phase 1 dose escalation and dose expansion study investigating TH-302 monotherapy in subjects with solid tumors. TH-302 monotherapy administered weekly for 3 weeks of an every 4 week cycle (Arm A) was initiated at a weekly dose of 7.5 mg/m^2 and the maximum tolerated dose (MTD) was established at 575 mg/m^2. Based on the toxicology studies, hematologic toxicity was expected to be the major dose-limiting adverse reaction of TH-302; however, hematologic toxicity has rarely been significant and dose-limiting toxicities were mucosal and skin toxicity. Dose-limiting toxicities (DLTs) of perianal and rectal ulcers and oral mucositis with associated dehydration were observed at 670 mg/m^2. Common adverse events include nausea, fatigue, vomiting, constipation and diarrhea. Skin toxicities including intertriginous erythema, desquamation and hyperpigmentation and mucosal toxicities including stomatitis and mucosal inflammation are also common. At TH-302 doses above 240 mg/m^2, prophylaxis against nausea and vomiting should be implemented using a regimen intended for moderately emetogenic chemotherapy. During the dose escalation, anti-tumor activity was observed in subjects with malignant melanoma, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Based on these data, a dose expansion cohort was opened at 575 mg/m^2 to enroll

subjects in these indications. Subsequently, to help determine the recommended Phase 2 dose, a dose expansion cohort was opened at 480 mg/m^2 to obtain safety and tolerability data and to obtain efficacy data in subjects with malignant melanoma, SCLC, hepatocellular cancer (HCC) and tumors with squamous or transitional cell histologies based on anti-tumor activity observed in TH-302 monotherapy and/or combination therapy in these indications. A separate treatment arm (Arm B) was opened to determine the MTD, DLTs, safety and preliminary anti-tumor activity utilizing a once every 3 week dosing regimen of TH-302. The Arm B dose was escalated from 670 mg/m^2 to 940 mg/m^2 and the MTD was established at 670 mg/m^2.

TH-CR-402 is a Phase 1/2 three-arm, multicenter, dose-escalation study to determine the MTD, DLT, safety and pharmacokinetics and to make a preliminary assessment of efficacy of TH-302 when used in combination with standard doses of gemcitabine, docetaxel, or pemetrexed. Subjects with advanced solid tumors who are candidates for therapy with one of the three cytotoxic chemotherapies were enrolled in the dose escalation portion of the study. The dose expansion portion of the study enrolled subjects with pancreatic cancer (gemcitabine arm), castrate-resistant prostate cancer (docetaxel) or NSCLC (docetaxel and pemetrexed). The starting dose of TH-302 in each of the treatment arms was 240 mg/m^2. The MTD of TH-302 in combination with weekly gemcitabine (1000 mg/m^2) was 340 mg/m^2. DLTs at higher TH-302 doses were Grade 3 esophagitis, Grade 3 pain due to perianal rash, Grade 4 neutropenia and Grade 4 thrombocytopenia. RECIST tumor response assessments have been reported for 34 subjects. One subject with locally advanced unresectable disease had a confirmed complete response. Eight subjects had a best response of partial response (four confirmed, three unconfirmed, one unconfirmed continuing on-study). An additional 22 subjects had stable disease. The stable disease or better rate was 91%. The MTD of TH-302 in combination with 75 mg/m^2 docetaxel was 340 mg/m^2. DLTs at higher TH-302 doses were Grade 4 neutropenia, Grade 3 infection with neutropenia and Grade 3 febrile neutropenia/Grade 3 diarrhea. The MTD of TH-302 in combination with 500 mg/m^2 pemetrexed was 480 mg/m^2. DLTs at higher TH-302 doses were Grade 3 stomatitis and Grade 4 thrombocytopenia.

TH-CR-403 is a Phase 1/2, multi center, dose escalation study to determine the safety, efficacy and PK of TH-302 in combination with full-dose doxorubicin in subjects with soft tissue sarcoma. In this study, the MTD of 300 mg/m^2 was established for the combination of TH-302 and 75 mg/m^2 doxorubicin with prophylactic growth factor support. DLTs at a TH-302 dose of 340 mg/m^2 were Grade 4 thrombocytopenia and Grade 3 infection with Grade 4 neutropenia. Enrollment was expanded at the MTD for subjects with advanced soft tissue sarcoma previously untreated with chemotherapy (neoadjuvant and adjuvant chemotherapy permitted). Tumor response data were available for 54 of the first 57 subjects and the best response rates were: partial response (18 of 54, 33%), stable disease (28 of 54, 52%) and progressive disease (8 of 54, 15%). The median progression-free survival was 6.4 months (95% CI: 5.6 months to 6.9 months). The 6-month progression-free rate was 56% and the 3-month progression-free

rate was 83%. The median overall survival had not been reached. The tumor response and stable disease rates and progression-free survival were all higher than those reported with single-agent doxorubicin. The combination regimen was well tolerated with acceptable

hematologic toxicity and manageable safety events.

Study TH-CR-404 is an open-label multi-center randomized controlled crossover Phase 2 study of two dose levels of TH-302 (240 and 340 mg/m^2) in combination with gemcitabine versus gemcitabine alone in patients with locally advanced unresectable or metastatic pancreatic adenocarcinoma. A total of 165 subjects are planned to be enrolled at a ratio of 1:1:1. Subjects on the gemcitabine arm who discontinue for disease progression and meet laboratory, tumor and performance status eligibility criteria may crossover to TH-302 plus gemcitabine at a randomized TH-302 dose of 240 mg/m^2 or 340 mg/m^2. TH-302 doses of 240 mg/m^2 or 340 mg/m^2 are administered IV over 30 minutes on Days 1, 8 and 15 of every 28-day cycle. The dose of gemcitabine is 1,000 mg/m^2 administered IV over 30 minutes on Days 1, 8 and 15 of a 28-day cycle. Gemcitabine administration will start 2 hours after completion of the TH-302 infusion when used in combination with TH-302. The primary efficacy endpoint is progression-free survival. Enrollment in the study was initiated in June of 2010.

Study TH-CR-407 is a Phase 1 open-label dose-escalation study with expansion at the MTD in subjects with advanced leukemias. TH-302 is administered as a 30-minute intravenous infusion daily for 5 days every 21 days. The starting dose is 120 mg/m^2. Subjects who successfully complete a 3-week treatment cycle without evidence of significant treatment-related toxicity or clinically significant progressive disease continue to receive treatment for up to six cycles. Enrollment in the study was initiated in June of 2010.

Study CTRC 11-24 (IND 112417) is a phase 1 dose escalation study in glioblastoma, with patients who were administered TH302 at a single preoperative dose of 575 mg/m^2. Once recovery from surgery was observed, patients received bevacizumab at the standard dose of 10mg/kg and TH302 at 240, 340, 480, or 670mg/m^2 given together in a Q2week schedule. In the final cohort of 670 mg/m^2, the surgical requirement was eliminated and patients were allowed to go directly to combination therapy with bevacizumab. No patients experienced a dose limiting toxicity within the first cycle at any dose level. The most common toxicity of any grade observed was anal mucositis, which was observed in 2 of 4 (50%) patients in the 480mg/m^2 cohort and 4 of 4 (100%) patients at 670 mg/m^2. The anal mucositis can be controlled with supportive care (see Appendix D). As 670 mg/m^2 has already been documented as the MTD when given every three weeks in single agent studies, no further dose escalation is planned. The phase 2 dose in combination with bevacizumab every 2 weeks is therefore 670 mg/m^2. While efficacy was not a primary endpoint of this study, two partial responses have been observed and 5 patients demonstrating stable disease, for a combined clinical benefit rate of 58% (n=12; with 2 not yet evaluable). Further, one patient has reached cycle 24 (day 640) and continues with stable disease. These observations suggest potential clinical activity of TH-302 in bevacizumab resistant glioblastoma.

1.1.5 Standard Treatment for Glioblastoma

Glioblastoma are highly aggressive tumors and invariably recur after standard of care first-line therapy. Currently, front-line treatment consists of a multi-modality approach that

includes maximal surgical resection, adjuvant radiation therapy with concurrent temozolomide at 75 mg/m^2 followed by 6 months of single-agent temozolomide at up to 200 mg/m^2. Temozolomide (a prodrug) is a rapidly and non-enzymatically converted to the active alkylating metabolite MTIC [(methyl-triazene-1-yl) – imidazole-4-carboxamide]. The cytotoxic effects of MTIC are manifested through alkylation of DNA at the O6, N7 guanine positions.

This multimodal approach has been the standard of care ever since the publication of the landmark EORTC phase III trial published by Roger Stupp et al in 2005 that demonstrated an improved primary end-point of median overall survival of this regimen when compared to adjuvant radiation alone. A median survival of 14.6 months was reported in the temozolomide group versus 12.1 months in the radiation alone group. At that time, radiation alone was considered standard of care in most countries. In the US, common practice was to add adjuvant nitrosurea-based chemotherapy regimen with a small survival benefit with significant adverse effects.

However, once a patient fails standard front-line therapy, prognosis is very poor and new therapies are needed. In the era of targeted therapies, anti-angiogenic drugs have come into the limelight. Bevacizumab, a recombinant humanized monoclonal antibody against VEGF, has been studied extensively over the past several years and has demonstrated rather impressive radiographic and clinical response rates when compared to historical data but the responses are not very long-lasting. The largest study to date was a phase II, multicenter, open-label, noncomparative trial conducted by Friedman et al in 2009 which evaluated the efficacy of bevacizumab (10 mg/kg every 2 weeks), alone and in combination with irinotecan (125 mg/m^2 in those non receiving enzyme-inducing anti-epileptic agents and 340 mg/m^2 in those who were taking these drugs), in patients with recurrent glioblastoma. In the bevacizumab-alone and the bevacizumab-plus-irinotecan groups, estimated 6-month progression-free survival rates were 42.6% and 50.3%, respectively; objective response rates were 28.2% and 37.8%, respectively; and median overall survival times were 9.2 months and 8.7 months, respectively. Nonetheless, these are all improved outcomes when compared to historical data in the recurrent setting.

Dose limiting toxicities of bevacizumab are hypertension, proteinuria, thromboembolic events, spontaneous bleeding and wound healing complications. In the phase II study conducted by Friedman, bevacizumab alone or bevacizumab plus irinotecan, 46.4% and 65.8%, respectively, experienced grade >or = 3 adverse events, the most common of which were hypertension (8.3%) and convulsion (6.0%) in the bevacizumab-alone group and convulsion (13.9%), neutropenia (8.9%), and fatigue (8.9%) in the bevacizumab-plus-irinotecan group. Intracranial hemorrhage was noted in two patients (2.4%) in the bevacizumab-alone group (grade 1) and in three patients (3.8%) patients in the bevacizumab-plus-irinotecan group (grades 1, 2, and 4, respectively). Bevacizumab obtained accelerated FDA-approval in 2009 for patients with recurrent glioblastoma multiforme.

1.1.6 Hypoxia Biomarkers

Hypoxia promotes more aggressive tumor phenotypes and has been associated with resistance to radiation and chemotherapies in GBM, as well as tumor invasion and poor patient survival. In particular, cells at pO_2 <10 mm Hg resist the ionizing effect of radiotherapy and cytotoxic effect of chemotherapy. Hypoxic necrotic foci with pseudopalisading tumor cells are one of the features that define GBM. A variety of methods have been devised to assess degree of hypoxia in xenografts and patient tumors.

Traditionally, the gold standard to measure hypoxia has been the use of a polarographic oxygen-sensitive probe, which provides direct measurement of tissue oxygen tension. However, this method has limitations, such as its inability to differentiate between viable and necrotic foci and is not practical in larger scale studies or in inaccessible tumors.

Other hypoxic markers that have been identified in pre-clinical studies include GLUT-1, HIF-1a, LDH-A and VEGF. Studies have also attempted to examine the spatial relationship between tumor hypoxia assessed by immunohistochemistry and ^{18}F-FDG and ^{18}F-FMISO auto-radiography but these have yet to receive regulatory approval.

The hypoxia-induced modulation of GLUT-1 and LDH-A also entails changes in cell metabolism, in particular glucose metabolism and lactate production. These metabolic changes, and in particular the modulation of lactate levels, provide valuable metabolic biomarkers that have the potential to be non-invasively imaged using magnetic resonance spectroscopic imaging (MRSI). Additional metabolic biomarkers of hypoxia have been investigated in various tumor types. Jiang and colleagues reported increased levels of choline-containing metabolites and lipid CH_3 colocalizing with the hypoxic areas of the tumor in a breast cancer model[14]. High resolution methods based on either MR spectroscopy or mass spectrometry platforms are also of value to obtain a detailed metabolic signature due to hypoxia in biopsy tissue or patient biofluids [15, 16].

1.1.7 Risks and Benefits

Phase I dose-escalation clinical studies involving TH-302 in combination with other cytotoxic agents (gemcitabine, docetaxel) have demonstrated dose limiting toxicities to be mostly hematological and dermatological. The MTD was 340 mg/m^2 when administered with gemcitabine and docetaxel. However, TH-302 increases the hematological toxicity of the chemotherapy. The TH-CR-401 phase I dose escalation study of TH-302 as a monotherapy in subjects with advanced solid tumors evaluated TH-302 given IV every week for 3 weeks of a 4 week cycle as well as TH-302 administered once every 3 weeks. The first dose-limiting toxicities were reported at weekly doses of 670 mg/m^2 and these were related to mucosal toxicities and not hematological.

It is important to note that TH-302 should not be administered to pregnant or lactating women since there is not enough data to determine teratogenicity or risks of breast feeding after receiving this drug. Animal studies suggest that TH-302 may have embryotoxic or teratogenic effects. Contraceptive measures should be applied during, and for at least six months after completion of therapy. Other potential sites of organ injury, include kidney, liver and GI function. The active metabolite of TH-302, Br-IPM, is an alkylating agent similar to the active metabolite of ifosphamide, IPM, therefore, renal tubular toxicity may

occur, especially if used concomitantly with other nephrotoxic drugs. There is no known antidote against TH-302.

Phase II clinical studies involving patients with recurrent glioblastoma treated with the anti-VEGF receptor monoclonal antibody, bevacizumab, have demonstrated improved radiographic response rates, based on traditional criteria, when compared to historical data. Most studies have administered this anti-angiogenic agent at a dose of 10 mg/kg IV every two weeks. Overall, this drug has been well tolerated. However, dose limiting toxicities that have been consistently reported include hypertension, proteinuria, thromboembolic events, spontaneous bleeding and wound healing complications.

The risk-to-benefit ratio is considered acceptable for both drugs to be used in this phase II clinical study based upon the patient population, the limited number of active agents for this aggressive cancer and the pre-clinical as well as clinical evidence of tumor activity and drug tolerability.

1.2 Rationale for the study

Clinical studies have demonstrated anti-tumor activity in patients treated with anti-angiogenic agents such as with bevacizumab, a recombinant human monoclonal antibody against VEGF receptor. VEGF has been found to be a critical regulator of angiogenesis. Response rates using bevacizumab have been reported to be between 20-50% in recurrent glioblastoma with a direct effect on vessel permeability. This direct vessel effect makes it difficult to interpret changes in contrast enhancement seen on brain MRI and assess whether these changes correlate with tumor burden after receiving bevacizumab. In many patients who recur after bevacizumab therapy, progression is manifest as a diffuse infiltrative pattern without worsening enhancement, resembling gliomatosis cerebri. Using a human GBM model in rats via xenografted tumors, Keunen et al, reported that anti-angiogenic treatment leads to loss of large-sized vessels resulting in reduced perfusion, blood volume and increase in hypoxia in the tumor microenvironment[16]. This increase in hypoxia leads to an increase in lactate production, stabilization of HIF-1 alpha and is accompanied by a dramatic increase in cell invasion into the normal brain.

Hypoxia is a powerful trigger for altered gene expression and has a multitude of tumorigenic properties, including, activation of specific pathways and transcription factors that control tumor stem cells. Hypoxia causes a shift to glycolytic metabolism, upregulates survival and growth factors, inhibits apoptosis, and stimulates the production of enzymes mediating invasiveness. This imbalance between oxygen delivery and oxygen consumption renders tumor cells with greater resistance to anticancer therapy such as radiation. Therefore, factors that regulate the hypoxic state represent potential targets for treatment of high grade glioma.

TH-302 is a pro-drug that when it is exposed to hypoxic conditions, it is reduced at the nitroimidazole site of the prodrug by intracellular reductases leading to the release of the alkylating agent Br-IPM. This active metabolite acts as a DNA crosslinking agent and is highly cytotoxic under hypoxic condition. The prodrug also diffuses to adjacent cells in normoxic regions, acting as a cytotoxic agent outside of the hypoxic activation zone. Pre-clinical studies have demonstrated that this drug is highly active in *in vitro* proliferation

assays in multiple human cancer cell lines. This tumor-selective hypoxia-activated pro-drug TH-302 has been shown to potentiate, *in vivo,* the anti-tumor efficacy of other anti-angiogenic agents such as sunitinib and sorafenib by targeting tumor hypoxia. These observations provide a translational rationale for the study of the combination of TH-302 with the anti-angiogenic anti-VEGF monoclonal antibody, bevacizumab.

This phase II study will enroll subjects with recurrent glioblastoma following disease progression while on bevacizumab. Since TH-302 remains intact as a prodrug in areas of normoxia toxicity is minimized.

1.3 Rationale for dose selection

TH-302

Phase I clinical studies have established the MTD of TH-302 given in a Q3week schedule at 670 mg/m^2 and when given in a Q1week schedule at 575 mg/m^2. In our Phase 1 investigator-initiated, dose-escalation study in glioblastoma, patients were administered TH302 at a single preoperative dose of 575 mg/m^2. Once recovery from surgery was observed, they received bevacizumab at the standard dose of 10mg/kg and TH302 at 240, 340, 480, and 670 mg/m^2 given together in a Q2week schedule. In the final cohort of 670 mg/m^2, the surgical requirement was eliminated and patients were allowed to go directly to combination therapy with bevacizumab.

No patients experienced a dose limiting toxicity within the first cycle at any dose level. Further, 670 mg/m^2 has already been documented as the MTD when given every three weeks in single agent studies. Therefore, based upon the lack of DLT in our Phase 1, the Phase 2 dose in combination with bevacizumab every 2 weeks is 670 mg/m^2.

Bevacizumab (Avastin)

Phase II clinical studies as well as the FDA have established the standard bevacizumab dose of 10 mg/kg IV every 2 weeks when treating patients with recurrent glioblastoma.

1.4 Rationale for selection of the subject population

There are no effective established treatments available after progression while on bevacizumab for patients with high-grade glioma. These patients invariably recur and have a poor prognosis. In this phase II study, patients with recurrent glioblastoma after bevacizumab and with no standard treatment options will be eligible for enrollment.

1.5 Rationale for *in vivo* Positron Emission Tomography Studies of Tumor Hypoxia

In order to generate in vivo measurements of hypoxia, subjects will be asked to participate in ^{18}F-Fluoromisonidazole (FMISO) PET scans, which have been used safely numerous times in humans since the 1980's [17]. FMISO is a nitromidazole family derivative, analogous to TH-302, which diffuses into cells and undergoes reduction in a hypoxic environment. This compound has also been used before to successfully image hypoxia in Grade IV astrocytomas [18,19]. Images will be corrected to background attenuation. The estimated average dose of ^{18}F-FMISO subjects will receive per scan is 250-300 MBq (range from 120-450 MBq). The scan will be initiated approximately 110-120 minutes post ^{18}F-

FMISO administration, and plasma pharmacokinetic samples (1mL) will be collected to assess decay of ^{18}F-FMISO.

1.6 Study Compliance

This study will be conducted in compliance with this protocol, the principles of Good Clinical Practices, and applicable regulations.

2. OBJECTIVES OF THE STUDY

2.1 Primary Objectives

1. To assess the safety of TH-302 in combination with bevacizumab for patients with high grade glioma
2. To determine the proportional progression-free survival (PFS) at 4 months for patients treated with combination bevacizumab and TH-302 following recurrence on single agent bevacizumab

2.2 Secondary Objectives

1. To evaluate overall survival of patients for patients treated with combination bevacizumab and TH-302 following recurrence on single agent bevacizumab
2. To assess preservation of Quality of Life (QOL) using the MD Anderson Symptom Inventory for Brain Tumors (MDASI-BT).

2.3 Exploratory Objectives

1. To explore the extent of tumor hypoxia and cerebral blood flow in vivo via ^{18}F-FMISO Positron Emission Tomography with correlation to patient response and survival
2. To explore the role of emerging biomarkers of hypoxia in predicting response to TH-302 and overall survival

3. STUDY DESIGN AND METHODS

3.1 Design

1. Multi-center, single-arm, two-stage prospective study, non-blinded
2. Combination therapy with bevacizumab at 10 mg/kg intravenously (IV) every 2 weeks and TH-302 at 670 mg/m2 IV every 2 weeks, in 6 week cycles, until disease progression.
3. 33 patients will be enrolled
4. TH-302 will be administered by IV infusion over 30-60 minutes on day 1, day 15, and day 29 of each 42 day cycle.
5. Bevacizumab will be administered by IV infusion on day 1, day 15, and day 29 of each 42-day cycle. The initial bevacizumab dose will be infused over 60

minutes. Infusion may be shortened to 30 minutes if the 60-minute infusion is tolerated well.

3.1.1 Investigational Sites

This study is a multi-center, single arm, two stage, open label trial. The only participating sites will be the CTRC @ UTHSCSA and Dana Farber Cancer Center. CTRC@UTHSCSA will implement ongoing monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring will occur before the clinical phase of the protocol begins, continue during protocol performance and through study completion. Additional monitoring practices may include but are not limited to; source verification of case report forms, review and analysis of the following: eligibility requirements of all participants , informed consent procedures, adverse events and all associated documentation, study drug administration/treatment, regulatory files, protocol deviations, pharmacy records, response assessments, and data management. Participating institutions will be required to participate in monthly teleconferences.

3.1.2 Statistics

3.1.2.1 Sample size justification

Subjects will be assessed on Day 1 of each 6 week cycle by MRI until disease progression according to RANO criteria[18] and as described below. This study will include up to 33 experimental subjects (plus an undetermined number of subjects rolling over from Protocol CTRC# 11-24) with an accrual interval of 12 months. There will be an additional follow up period of 12 months. Based on historical data, the median progression free survival of 54 patients on control salvage therapy was 37.5 days (Quant et al 2008). If the true median progression-free survival in the historical control group and the experimental treatment group treated at the MTD are 37.5 days and 67 days, then assuming a one-parameter exponential model, the corresponding mean times to progression are 54.1 [=37.5/log(2)] and 96.7 [=67/log(2)] days, and the proportion progression-free at 4 months is estimated to be 0.289 in the experimental group and 0.109 in the historical control group. With one-sample 2-stage testing and alpha=0.05 and 80% power, the optimal 2-stage design will enroll 11 subjects in the first stage. If 1 or fewer subjects are progression-free at 4 months then the trial will be terminated. If the trial goes to the second stage, then a total of 33 subjects will be studied. If the total number progression-free at 4 months is less than or equal to 6 then the test drug is rejected [PASS Version 11, NCSS, Kaysville, Utah].

3.1.2.2 Methods

The numbers of patients screened, screen failures by reason, the number enrolled and completing the study at each stage and the number and proportion progression-free at 4 months at each stage will be tabulated. The distribution of time to progression and death will be summarized with Kaplan-Meier curves. If the trial goes to the second stage, the

hypothesis $H_o:\theta=\theta_C$ versus $H_o:\theta\neq\theta_C$, where θ and θ_C are the mean times to disease progression of the experimental and historical control groups, will be contrasted with a one-sample two-sided log rank statistic. Analyses of the relation between hypoxia and cerebral blood flow in vivo, between hypoxia and selected biomarkers and between hypoxia and quality of life will be exploratory and descriptive. Adverse events will be tabulated. All statistical testing will be two sided with a significance level of 5%. SAS Version 9.3 for Windows (SAS Institute, Cary, North Carolina) will be used throughout. Statistical analyses will be performed by Joel E. Michalek, PhD, Department of Epidemiology and Biostatistics University of Texas Health Science Center at San Antonio.

3.1.3 Treatment Regimens

3.1.3.1 TH-302

TH-302 will be administered intravenously over 30-60 minutes at 670 mg/m^2 on Days 1, 15 and 29 of each 42-Day (6 week) cycle.

Dose of TH-302 is to be re-calculated only if a subject's body weight changes by >10% from baseline.

Each dose will be prepared in D5W for injection in non-DEHP containing infusion bags and administered intravenously via an infusion pump through a non-DEHP containing IV administration set (See Section 6.3 for details). The full recommended infusion volume should be used. When possible, TH-302 should be administered through a central line. Detailed instructions for dilution and administration are provided in the Pharmacy Manual.

Prophylaxis against nausea and vomiting should be applied using a regimen intended for moderately emetogenic chemotherapy.

3.1.3.2 Bevacizumab

Bevacizumab will be administered intravenously at 10 mg/kg on day 1, day 15, and day 29 of each cycle.

3.1.3.3 Dose Modifications

Dose modification rules should be followed for all hematologic and non-hematological toxicity regardless of causality and for any other toxicity that is not clearly related to disease progression, intercurrent illness, concomitant medications or other non-drug intervention.

Please follow the package insert for all bevacizumab dose modifications and discontinuations.

- If a subject requires more than 2 dose level reductions for non-hematological toxicity, he/she should discontinue from the study.
- Any subject who misses one complete cycle or treatment-related toxicity should discontinue from the study.

3.1.4.1 TH-302 Dose Modifications

- If Day 1 of a cycle for either drug must be withheld, the whole cycle should be delayed.
- At Day 15 or Day 29, if both drugs must be withheld, the dose should be skipped.
- At Day 15 or Day 29, if only one drug must be withheld, that drug's dose should be skipped rather than delayed and the other drug may be given alone.
- Dose modifications for hematologic toxicity should be independently assessed at each visit as described on Table 3.1-1.
- Prophylactic G-CSF may be implemented in subsequent cycles if neutropenia results in dose reduction or dose delay at prior doses. Therapeutic use of hematopoietic colony-stimulating factors is permitted following ASCO guidelines.
- G-CSF may be implemented in the management of neutropenia to avoid dose reductions or holding a dose if neutropenia recovers within 48 hours

Hemoglobin must be ≥9 g/dL at Cycle 1/Day 1 and must be ≥8 g/dL for all subsequent doses.

Table 3.1-1 TH-302 dose modifications for hematologic toxicity

			% of Full Dose for any Cycle	
ANC (/μL)		Platelets (/μL)	Day 1	Days 15 or 29
≥1500	AND	≥100,000	100	100
1000-1499	AND	≥75,000-99,999	75	100
500 – 999	OR	50,000-74,999	Hold	75
<500	OR	<50,000	Hold	Hold

Follow Table 3.1-2 for TH-302 dose modifications for non-hematologic toxicity.

- For bevacizumab dose modifications should follow the manufacturer's labeling.
- All treatment related toxicities will be documented and graded based on NCI CTCAE v4.03.
- Management of toxicity is as directed under section 7 and per algorithms for management of hand-foot skin reactions, anal mucositis, oral mucositis, injection site reaction, and hyperpigmentation listed in Appendix D.

Table 3.1-2 TH-302 Dose Modifications for Non-Hematologic Toxicity

		TH-302	
Toxicity	**Details**	**Hold Dose**	**% of Dose after Recovery**

Grade 2 or 3 Bilirubin	Regardless of causality	Hold dose until resolution to Grade 0 or 1	100
Grade 2	except for elevated ALT/AST, nausea, vomiting, diarrhea, alopecia and fatigue	Hold dose until resolution to Grade 0 or 1	100
	Intolerable skin toxicity[1]	Hold dose until resolution to Grade 0 or 1	75
Grade 3	Except for elevated ALT/AST, nausea and vomiting	Hold dose until resolution to Grade 0 or 1	75
Grade 3	Nausea	Hold dose until resolution to ≤ Grade 2	100
Grade 3	Vomiting and AST/ALT elevation	Hold dose until resolution to ≤ Grade 1	100
Grade 4	Life-threatening conditions (study drug-related)	Treatment should be discontinued	NA

[1]Please see Appendix D for algorithms for management of hand-foot skin reactions, anal mucositis, oral mucositis, injection site reaction, and hyperpigmentation.

Any subject who misses more than one cycle for treatment-related toxicity should discontinue from the study. All treatment related toxicities will be documented and graded based on NCI CTCAE v4.03.

4. PATIENT POPULATION

4.1 Inclusion Criteria

1. At least 18 years of age
2. Ability to understand the purposes and risks of the study and has signed a written informed consent form approved by the investigator's IRB/Ethics Committee

3. Histologically confirmed glioblastoma
4. Progression following both standard combined modality treatment with radiation and temozolomide chemotherapy, as well as bevacizumab
5. Recovered from toxicities of prior therapy to grade 0 or 1
6. ECOG performance status ≤ 2
7. Life expectancy of at least 3 months
8. Acceptable liver function:

a. Bilirubin ≤ 1.5 times upper limit of normal
b. AST (SGOT) and ALT (SGPT) ≤ 3.0 times upper limit of normal (ULN);

9. Acceptable renal function:
   a. Serum creatinine ≤ULN
10. Acceptable hematologic status (without hematologic support):
    a. ANC ≥1500 cells/uL
    b. Platelet count ≥100,000/uL
    c. Hemoglobin ≥9.0 g/dL
11. All women of childbearing potential must have a negative serum pregnancy test and male and female subjects must agree to use effective means of contraception (surgical sterilization or the use or barrier contraception with either a condom or diaphragm in conjunction with spermicidal gel or an IUD) with their partner from entry into the study through 6 months after the last dose

4.2 Exclusion Criteria

1. The subject is receiving warfarin (or other coumarin derivatives) and is unable to switch to low molecular weight heparin (LMWH) before the first dose of study drug.
2. The subject has evidence of acute intracranial or intratumoral hemorrhage either by MRI or computerized tomography (CT) scan. Subjects with resolving hemorrhage, punctate hemorrhage, or hemosiderin are eligible.
3. The subject is unable to undergo MRI scan (eg, has pacemaker).
4. The subject has received enzyme-inducing anti-epileptic agents within 14 days of study drug (eg, carbamazepine, phenytoin, phenobarbital, primidone).
5. The subject has not recovered to National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03 Grade ≤ 1 from AEs (except alopecia, anemia and lymphopenia) due to surgery, antineoplastic agents, investigational drugs, or other medications that were administered prior to study drug.
6. The subject has evidence of wound dehiscence
7. Severe chronic obstructive or other pulmonary disease with hypoxemia (requires supplementary oxygen, symptoms due to hypoxemia or oxygen saturation <90% by pulse oximetry after a 2 minute walk) or in the opinion of the investigator any physiological state likely to cause normal tissue hypoxia
8. The subject is pregnant or breast-feeding.
9. The subject has serious intercurrent illness, such as:
   a. hypertension (two or more blood pressure [BP] readings performed at screening of > 150 mmHg systolic or > 100 mmHg diastolic) despite optimal treatment
   b. non-healing wound, ulcer, or bone fracture
   c. significant cardiac arrhythmias
   d. untreated hypothyroidism
   e. uncontrolled active infection
   f. symptomatic congestive heart failure or unstable angina pectoris within 3 months prior study drug
   g. myocardial infarction, stroke, transient ischemic attack within 6 months
   h. gastrointestinal perforation, abdominal fistula, intra- abdominal abscess within

1 year
   i. history or clinical evidence of pancreatitis within 2 years
10. The subject has inherited bleeding diathesis or coagulopathy with the risk of bleeding.
11. The subject has received any of the following prior anticancer therapy:
    a. Non-standard radiation therapy such as brachytherapy, systemic radioisotope therapy, or intra-operative radiotherapy (IORT). Note: stereotactic radiosurgery (SRS) is allowed
    b. Non-bevacizumab systemic therapy (including investigational agents and small-molecule kinase inhibitors) or non-cytotoxic hormonal therapy (eg, tamoxifen) within 7 days or 5 half-lives, whichever is shorter, prior first dose of study drug
    c. Biologic agents (antibodies, immune modulators, vaccines, cytokines) within 21 days prior to first dose of study drug, with the exception of bevacizumab which can be 14 days or maintain the subjects current bevacizumab dosing schedule
    d. Nitrosoureas or mitomycin C within 42 days, or metronomic/protracted low-dose chemotherapy within 14 days, or other cytotoxic chemotherapy within 28 days, prior to first dose of study drug
    e. Prior treatment with carmustine wafers
    f. Prior treatment with TH-302

5. PROCEDURES

5.1 SUMMARY OF PROCEDURES

Subjects are expected to participate with continued follow up for survival until one year after the last dose of study drug. Please refer to Appendix A, Schedule of Assessments, for an overview of the study assessments. Subjects who withdraw from the study before all follow-up procedures have been performed will be managed and documented as described in Section 8, Removing Subjects from the Study.

A summary of visits and clinical procedures is found in Appendix A, Schedule of Assessments. The total duration of the active part of the study for each subject will be approximately 30 weeks, divided as follows:

- Up to 3 weeks predose (screening period)
- Up to four 6-week treatment periods of combined TH-302 and bevacizumab.
- Study termination visit 1-2 weeks after last dose of study medication

When a subject has completed the study termination or early termination visit, he/she and/or a family member will be contacted for survival information every 3 months until one year from last dose.

5.2 SCREENING PROCEDURES

All subjects will be screened within 21 days prior to Cycle 1 Day 1. Vital signs, clinical

laboratory test results, weight and AEs will be used to assess safety. Efficacy will be assessed based on tumor assessments (objective response rate, progression-free survival and duration of response) conducted at intervals during the study. Subjects who have not progressed after 4 cycles will be permitted to continue therapy..

During screening, candidates for the study will be fully informed about the nature of the study and possible risks, and will receive a copy of the informed consent for review. Candidates must read the consent form and sign the document after the investigator has answered all questions to the candidate's satisfaction. Further procedures can begin only after the consent form has been signed. The original signed consent form will be retained by the investigator and a copy will be given to the candidate. Candidates will be evaluated for entry into the study according to the stated inclusion and exclusion criteria (Section 4, Study Population). The investigator will evaluate the results of all examinations, including clinical laboratory tests, and will determine each candidate's suitability for the study. The investigator must know the baseline results before enrollment. The pregnancy test for females of reproductive potential must be negative for those subjects to proceed to enrollment. All screening procedures must be done within 21 days of Cycle 1 Day 1, unless otherwise specified. The following procedures will be performed to establish each candidate's general health and qualifications for possible enrollment into the study:

- Obtain signed, written informed consent and permission to use protected health information, (in accordance with the Health Insurance Portability and Accountability Act or HIPAA). Refusal to sign informed consent and permission excludes an individual from the study.
- Record medical history, including cancer history: histology of primary tumor (including degree of differentiation), date of cancer diagnosis, types and dates of prior anti-tumor therapy (including surgery, radiation therapy, systemic therapy), and date of most recent disease progression.
- Record recent medication history, including vitamins, herbal preparations, blood products, and other over the counter (OTC) drugs.
- Record blood pressure (BP), heart rate (HR), respiratory rate (RR) and temperature measurements. In subjects with known significant pulmonary disease, measure oxygen saturation using pulse oximeter after a 2 minute walk

- Perform a complete physical examination, including height and weight.
- Perform tumor assessment with MRI of the brain per RANO criteria (refer to Appendix C)
- Assess Eastern Cooperative Oncology Group (ECOG) Performance Status score (see Appendix B, Eastern Cooperative Oncology Group Performance Status Scale).
- Draw blood samples for hematology, chemistry and coagulation (if clinically indicated)
- Obtain a blood sample for serum HCG pregnancy test in female subjects of child-

bearing potential (all female subjects unless surgically sterilized or at least 1 year post-menopausal).

- Obtain a urine sample for urinalysis with micro.
- Obtain a baseline ECG to assess for cardiac arrhythmias or evidence of recent cardiac events.
- Review inclusion and exclusion criteria (see Section 4, Study Population).

5.3 TREATMENT PERIOD ALL CYCLES

Study drug should be administered within ±2 days of the nominal time point. Lab tests used for determining dosing must be done within 5 days before the first dose of study drug (Cycle l/Day 1), within 3 days before Days 15 and 29 of Cycle 1, and Days 1, 15, and 29 of all subsequent cycles. All other required study assessments should be obtained within 5 days of the nominal time point unless otherwise specified. Subjects must receive the first dose of study drug within 21 days of the start of screening.

5.3.1 Pretreatment Evaluations and Procedures

The following procedures will be done in all subjects**:**

- If screening assessments have not been performed within 5 days of Cycle 1 Day 1, then screening assessments will be repeated with the exception of informed consent, demographics, MRI, medical/surgical history, and ECG;
- Serum and whole blood for biomarker analysis will be collected from patients on screening and immediately prior to treatment on Day 1 of each cycle.
- Baseline PET imaging will be performed within 3 days prior to Cycle 1 Day 1

5.3.2 Procedures (Day 1 of each cycle)

Before administering TH-302, the following procedures will be done in all subjects within 5 days of Cycle 1 Day 1:

- Record interim medical history since screening;
- Confirm that subject continues to meet inclusion/exclusion criteria;
- Record concomitant medications for previous 14 days;
- Draw serum and whole blood samples for biomarker analysis

The following procedures will be done predose on all subjects within a 5 day window prior to Cycle 1 Day 1 and within 3 days of Day 1 for **All Future Cycles** (unless otherwise specified) and before administering TH-302 in subjects:

- Assess Eastern Cooperative Oncology Group (ECOG) Performance Status score;
- Assess whether subject is adequately hydrated for administration of study drugs
- Cycle 2 and all subsequent cycles: Record AEs since last visit;
- Any skin or mucosal lesions considered due to TH-302 should be photographed;
- Record concomitant medications since last cycle;

- Record weight and vital signs;
- Detailed physical exam including neurologic assessment;
- Draw blood samples for hematology, chemistry, and coagulation (if clinically indicated);
- Draw serum and whole blood samples for biomarker analysis;
- Obtain (serum or urine) pregnancy test for females of childbearing potential prior to start of Cycle 1, and at every other cycle thereafter;
- Urine dipstick for protein and glucose. If positive and a change from baseline or previous cycle, complete urinalysis including microscopic analysis should be performed;
- Triplicate ECG measurements will be done within 30 minutes prior to infusion of TH-302 and within 30 minutes of the end of infusion of TH-302 (Cycle 1 only);
- Administer TH-302 (see section 3.1.4.1 for details) (Plus or minus 2 days.);
- Administer bevacizumab (see section 3.1.4.2 for details). (Plus or minus 2 days.).

5.3.3 Procedures (Days 15 and 29 of each cycle)

- A physical exam will be done on D15 of Cycles 1 and 2. This may occur up to 3 days prior to Day 15;
- Physical exam on D15 for Cycle 3 and beyond will *only* be done if clinically indicated. If done, this may occur up to 3 days prior to Day 15;
- Physical exam will be done on every Day 29 visit (This may occur up to 3 days prior to Day 29);
- Record concomitant medications drugs since the last visit. This may occur up to 3 days prior to Days 15 and 29;
- Record AEs since the last visit. This may occur up to 3 days prior to Days 15 and 29;
- Urine dipstick for protein and glucose. If positive and a change from baseline or previous cycle, complete urinalysis including microscopic analysis should be performed;
- Measure and record vital signs (BP, HR, RR, temperature). This may occur up to 3 days prior to Days 15 and 29;
- Triplicate ECG measurements will be done within 30 minutes prior to infusion of TH-302 and within 30 minutes of the end of infusion of TH-302 (Cycle 1, Day 15 only);
- Obtain blood samples for hematology, chemistry. This may occur up to 3 days prior to Days 15 and 29;
- Administer TH-302 (see section 3.1.4.1 for details). (Plus or minus 2 days.);
- Administer bevacizumab (see section 3.1.3.2 for details). (Plus or minus 2 days.).

5.3.4 F-MISO PET Imaging Procedures

- F-MISO PET imaging will be performed within 3 days of the first dose of TH-302.

- Prior to Cycle 2 Day 1, F-MISO PET imaging will be performed in select individuals following review of initial imaging results. This imaging will be optional.
- Plasma pharmacokinetic samples (1ml) will be collected to assess the decay of 18F-MISO

5.4 STUDY TERMINATION/EARLY STUDY TERMINATION AND SURVIVAL FOLLOW-UP

5.4.1 Study Termination / Early Study Termination

This visit will occur 1-2 weeks after the last dose of TH-302 treatment for subjects who terminate. The following will be done at the Termination/Early Termination visit:

- Record concomitant medications, including vitamins, herbal preparations, blood products, and other OTC drugs since the last visit;
- Record AEs since the last visit;
- Perform a complete physical examination, including weight;
- Assess Eastern Cooperative Oncology Group (ECOG) Performance Status score;
- Measure and record vital signs (BP, HR, RR, temperature);
- Obtain blood samples for hematology, and chemistry
- Obtain serum and whole blood samples for biomarker analysis;
- Obtain a blood sample for serum HCG pregnancy test in female subjects of child-bearing potential (all female subjects unless surgically sterilized or at least 1 year post-menopausal);
- Obtain a urine sample for urinalysis with micro; and
- Perform tumor assessments, using the same imaging assessments done at baseline, if not done within past 6 weeks.

In accordance with good medical practice, any ongoing study drug-related AE present at study termination, including a clinically significant laboratory test abnormality, will be followed until resolved or until the event stabilizes and the overall clinical outcome has been ascertained. Adverse events starting up to 30 days after the last dose of study medication may be collected by telephone contacts.

5.4.2 Survival Follow-up

When a subject has completed the study termination or early termination visit, he/she and/or a family member will be contacted for survival information every 3 months until one year from the last dose. Anti-tumor therapy (description and dates) since the last contact will be collected at each survival follow up.

5.5 SAFETY PROCEDURES

5.5.1.1 Physical Examination

A complete physical examination will be performed at screening and at study termination or early study termination and results will be recorded by the investigator (or designee). Limited physical examination will be done within 5 days before Day 1 of each cycle. Body

weight will be measured on Day 1 of every cycle. The results of the physical examinations will be used for safety monitoring purposes only. At each study visit, according to good medical practice, the subject's general health (e.g., appearance, adequacy of hydration, presence of illness or injury, temperature, and vital signs indicative of a concurrent illness) will be assessed to determine whether continued dosing is appropriate.

5.5.1.2 Vital Signs

BP, HR, RR and temperature will be measured at the following time points:

- Screening
- Cycles 1-4
- All subjects: Day 1, Day 15, and Day 29 of every cycle (predose and postdose for each study drug administered)
- Study Termination or Early Study Termination

Blood pressure and HR measurements should be obtained with the subject's arm unconstrained by clothing or other material. The measurements will be obtained with the appropriate cuff size from the opposite arm from that used for blood sampling, where possible, which is supported at the level of the heart. All BP measurements will be obtained from the same arm throughout the dosing period. The cuff should be placed on the designated arm at least 10 minutes prior to taking BP measurements.

5.5.1.3 Disease Assessment

Patients will be assessed at screening, within 3 days prior to the first dose, and at the end of every cycle, with MRI per the modified RANO criteria as detailed in Appendix C.

6. MATERIALS AND SUPPLIES

The following instructions are for the study drug, TH-302.

6.1 Supplies

Liquid Drug Product: TH-302 injection will be supplied in a 10 mL glass vial with a rubber stopper and aluminum crimped seal. Each vial contains 650 mg TH-302 (100 mg/mL). Each vial of study medication will be labeled clearly with information as required by institutional policy which may include: disclosing the lot number, route of administration, Sponsor's name, and appropriate, required precautionary labeling.

6.2 Storage

Liquid Drug Product: TH-302 injection must be stored in a secure area with limited access under controlled conditions as outlined in the TH-302 pharmacy manual.

6.3 Dose Preparation and Method Of Administration

Liquid Drug Product: Each dose will be prepared in a non-DEHP containing 5% Dextrose in Water (D5W) for injection for clinical use infusion bag and administered intravenously via an infusion pump and non-DEHP containing IV administration set. Detailed instructions for dilution are provided in the Pharmacy Manual.

The following table outlines the dilution volume and infusion time based on total dose administered.

Total TH-302 Dose (mg)	Infusion Volume (mL)	Infusion Duration (minutes)
< 1000	500	30-60
≥ 1000	1000	60

6.4 Drug Accountability

The investigator is responsible for the control of drugs under investigation. Adequate records of the receipt and disposition of all study drug shipped to the site must be maintained. Records will include dates, quantities received, quantities dispensed, and the identification codes of the subjects who received study drug. The individual administering the study drug will write the study number, subject number, date, and start/stop times of administration on the study drug label, and the Drug Accountability Record, as appropriate.

6.5 Disposition of Used and Unused Vials of Study Medication

All used and partially used vials will be destroyed by the pharmacy per the site's standard institutional procedures and in accordance with local and federal regulations as applicable. Periodically throughout and at the conclusion of the study, inventory checks and accountability of study materials will be conducted by a representative of Threshold or its designated agent. All unused vials must be retained by the pharmacy until such time they receive approval from Threshold or its designated agent to ship unused vials back to the sponsor or the sponsor instructs the site to destroy the unused vials. At the end of the study, a final drug accountability will be performed in accordance with the site's institutional procedures. The final Drug Accountability and Drug Destruction Record(s) will be retained on site which will include the date, lot number, and quantities returned to Threshold or its designated agent or destroyed locally.

The investigator's copy of the Drug Return Destruction Record(s) must accurately document the destruction of all study drug supplies. Records will also include dates, lot numbers, and quantities returned to Threshold or its designated agent or destroyed locally.

7. MANAGEMENT OF INTERCURRENT EVENTS

Comprehensive assessments of any apparent toxicity experienced by the subject will be performed throughout the course of the study. Study site personnel will report any clinical AE, whether observed by the investigator or reported by the subject.

7.1.1 Grading of Toxicity

Clinical AEs or abnormal laboratory test results will be assessed by the principal investigator or other designated other physician, in accordance with the CTCAE v4.03 criteria (available at http://evs.nci.nih.gov/ftp1/CTCAE/About.html).

7.1.2 Monitoring and Treatment of Toxicity

A physician or other qualified medical professional (e.g. Physician Assistant, Nurse Practitioner) designated by the Principal Investigator will manage and treat any toxicity. Specific algorithms for management of hand-foot skin reactions, anal mucositis, oral mucositis, injection site reaction, and hyperpigmentation are included in Appendix D. Subjects should be referred to an ophthalmologist for evaluation if clinically significant ophthalmologic abnormalities are noted. The ophthalmologist should be directed to perform examinations as clinically indicated.

7.2 Adverse Events

A physician or other qualified medical professional (e.g. Physician Assistant, Nurse Practitioner) designated by the Principal Investigator will assess the seriousness, severity, and causality of an AE based on the following definitions.

7.2.1 Defining Adverse Events

An adverse event (AE) is any undesirable event occurring to or in a subject enrolled in a clinical trial, whether or not the event is considered related to the study drugs (TH-302, bevacizumab). This includes the time periods beginning after the first administration of study drug until 30 days after the last dose of study drug.

Adverse events include the following types of occurrences:

1) Suspected adverse reactions;
2) Other medical experiences, regardless of their relationship to the study drug, such as injury, causes for surgery, accidents, increased severity of pre-existing symptoms, apparently unrelated illnesses, and significant abnormalities in clinical laboratory values, physiological testing, or physical examination findings; and
3) Reactions from drug overdose, abuse, withdrawal, sensitivity, or toxicity.

7.2.1.1 Serious Adverse Events

A serious adverse event (SAE) is any adverse' experience that occurs at any dose and results in any of the following outcomes.

1) **Death.** This includes any death that occurs during the conduct of the clinical study, including deaths that appear to be completely unrelated to the study drug (e.g., car accident). However, deaths that occur due to disease progression are not considered SAEs, but should be reported as a death on study. If a subject dies during the study, and an autopsy is performed, the autopsy results should be sent to Threshold. Possible evidence of organ toxicity and the potential relationship of the toxicity to the study drug are of particular interest. The autopsy report should distinguish between the relationship between the underlying diseases, their side effects, and the cause of death.

2) **Life-threatening adverse experience.** This includes any AE during which the subject is, in the view of the investigator, at immediate risk of death from the event as it occurs. This definition does not include any event that may have caused death if it had occurred in a more severe form.

3) Persistent or significant disability or incapacity

4) Inpatient hospitalization or prolongation of existing hospitalization

5) Congenital anomaly or birth defect

6) Other medically important event which, according to appropriate medical judgment, may require medical or surgical intervention to prevent one of the outcomes listed above.

7) Pregnancy occurring in subjects treated with TH-302 should be reported using the serious adverse event reporting form.

7.2.1.2 Nonserious adverse events

A nonserious AE includes any AE that is not defined as an SAE.

7.2.1.3 Unexpected adverse events

An unexpected AE is any AE that is not identified in nature, severity or frequency.

7.2.2 Documenting All Adverse Events

Record all AEs as descriptive findings (symptoms, or laboratory, physical exam, or vitals abnormalities) or diagnoses if etiology is known. Included are all AEs that occur after the start of treatment or within 30 days of administration of the last dose of study drug. Record AEs of any severity and AEs that are assessed as serious or not serious.

Note: Unchanged, chronic conditions and cancer symptoms present at baseline are NOT AEs and should not be recorded unless there is an exacerbation or worsening in severity of a chronic condition or cancer symptom after the first administration of study drug until 30 days after the last dose of study drug. Chronic conditions and/or cancer symptoms that exacerbate or worsen in severity should be documented as a "worsening" condition. Death due to disease progression and measures of disease progression collected as efficacy endpoints (eg increasing tumor size or new lesions) are not considered adverse events, but should be collected as termination reasons (if applicable) and/or noted in tumor assessment appropriate. Other reasons for death occurring during the AE reporting period are SAEs and should be reported as such.

7.2.2.1 Grading of Adverse Events

Severity of AEs or clinically significant laboratory test results will be assessed in accordance with the grading scale presented in the Common Toxicity Criteria for Adverse Events (CTCAE) version 4.03. A copy of this document can be found at the following internet site: http://evs.nci.nih.gov/ftp1/CTCAE/About.html. Clinically significant abnormal laboratory results and lab results requiring an intervention will be recorded as AEs and should describe whether the lab result was increased or decreased. The following definitions for rating severity of AEs will be used for events not covered in the CTCAE.

Grade 1: Mild; awareness of signs or symptoms that are easily tolerated, are of minor irritant type, cause no loss of time from usual activities, do not require medication or further medical evaluation, and/or are transient.

Grade 2: Moderate; signs or symptoms sufficient to interfere with function but not

activities of daily living.

Grade 3: Severe; signs or symptoms sufficient to interfere with activities of daily living; signs and symptoms may be of a systemic nature, or require further medical evaluation and/or treatment.

Grade 4: Disabling or with life-threatening consequences. (This definition does not include any event that might have caused death if it had occurred in a more severe form.)

Grade 5: Death

7.2.2.2 Relationship to Study Drug

Using the following criteria, investigators will assess whether there is a reasonable possibility that the study drugs (TH-302, bevacizumab) caused or contributed to the AE.

Yes

The time sequence between the onset of the AE and study drug administration is consistent with the event being related to study drug; and/or There is a possible biologic mechanism for study drug causing or contributing to the AE; and the AE may or may not be attributed to concurrent/underlying illness, other drugs, or procedures.

No

Another cause of the AE is most likely; and/or the time sequence between the onset of the AE and study drug administration is inconsistent with a causal relationship; and/or a causal relationship is considered biologically unlikely.

7.2.2.3 Abnormal Laboratory Test Results as Adverse Events

The investigator will monitor the laboratory test results and determine the clinical significance of any result that falls outside of the reference range. In accordance with good medical practice, any clinically significant abnormal laboratory test results must be followed until resolved or stabilized. Abnormal laboratory test results should not be reported as AEs unless, in the opinion of the investigator, the results constitute or are associated with a clinically relevant condition or require intervention.

In the event of unexplained, clinically significant abnormal laboratory test results, the tests should be repeated immediately and followed up until the values have returned to within the reference range or to baseline for that subject.

7.2.3 Reporting and Documenting Serious Adverse Events

Serious adverse events (SAE) that occur at any time point after the first dose of study drug until 30 days after the last dose of study drug must be reported. SAEs must be reported as per institutional policy and as required under the Data Safety Monitoring Plan (see Section 10.6 for DSMP). Contact information for SAE reporting to CTRC@UTHSCSA will be provided to each study site.

Submit all known subject information (listed below) within 24 hours of knowledge of the

SAE occurrence. The following information should also be entered in the database (or as much as possible to obtain and still report the event within 24 hours):

a) Subject's Demographic Data

b) Subject's weight

c) Description of SAE, including date of onset and duration, severity, and outcome

d) All dosing data of study drugs administered up to the date the SAE occurred

e) Action taken regarding study drug administration

f) Relationship of SAE to study drugs

g) Concomitant medications, including regimen and indication

h) Intervention, including concomitant medications used to treat SAE

i) Pertinent laboratory data and diagnostic tests conducted and date

j) Pertinent medical history of subject

k) Date of hospital admission discharge (if applicable)

l) Date of death (if applicable)

2) Perform appropriate diagnostic tests and therapeutic measures, and submit all follow-up substantiating data, such as diagnostic test reports and autopsy report to the study PI.

3) Conduct appropriate consultation and follow-up evaluations until the events are resolved, stabilized, or otherwise explained by the principal investigator.

4) Review each SAE report and evaluate the relationship of the SAE to study treatment and to the underlying disease. The study PI will determine whether the SAE is unexpected in nature.

5) Based on a cooperative assessment of the SAE between the treating physician and the study PI, a decision for any further action will be made. The primary consideration is subject safety. If the discovery of a new SAE related to the study drug raises concern over the safety of its continued administration to subjects, the PI will consult with Threshold Pharmaceuticals and will take immediate steps to notify the FDA.

6) The investigator must report all SAEs and unexpected problems promptly to his or her IRB/IEC, as appropriate (see ICH Guidelines, Good Clinical Practice [E6]).

Other actions regarding SAEs might include the following:

a) Protocol amendment

b) Discontinuation or suspension of the protocol

c) Modification of informed consent to include recent findings

d) Informing current study participants of new findings

e) Identification of specific AEs as drug-related

7.2.4 Follow Up of Adverse Events

All AEs are followed until they are resolved or determined to be irreversible or otherwise explained by the principal investigator.

7.3 CONCOMITANT AND EXCLUDED THERAPY

All medications and blood products (prescription and over-the-counter including herbal preparations) taken within 14 days of Cycle l/Week 1 will be recorded by the investigator (or designee). The reason(s) for treatment, dosage, and dates of treatment should be recorded in the source documents. In addition, concomitant medications used to treat adverse events occurring up to 30 days after the last dose of study drug will be recorded.

Female subjects who have been on hormone replacement therapy (HRT) for menopausal symptoms for a period of at least 2 months will not be excluded from the study provided the HRT regimen remains unchanged during the conduct of the study.

8. REMOVING SUBJECTS FROM THE STUDY

8.1 Criteria for Termination

Subjects are free to discontinue (withdraw) at any time during this clinical trial. If a subject withdraws from participation in the study during the treatment period, he or she should be encouraged to return for an early termination visit for evaluation of safety (see Section 5.4.1, Study Termination/Early Study Termination).

The investigator has the right to discontinue any subject from study drug administration or study participation. Reasons for subject discontinuation may include, but are not limited to, the following:

a) Clinically significant deterioration of the subject’s condition;

b) Disease progression;

c) Requirement for other anti-tumor therapy during the study;

d) Noncompliance;

e) Pregnancy;

f) Significant AE;

g) Subject's right to withdraw from the study at any time, with or without stated reason;

h) Significant protocol violation;

i) Lost to follow-up;

j) Death;

Any other reason that, in the opinion of the principal investigator, would justify the removal of a subject from the study.

The primary consideration in any determination to discontinue a subject's participation must be the health and welfare of the subject.

All subjects will be instructed on the importance of complying with the requirements of the study. It is expected that subjects will complete all of the necessary visits. If a subject does not return for follow-up visits as directed or does not adhere to the study requirements, the investigator will determine if early withdrawal should occur.

8.2 Documentation

The primary reason for early removal of a subject from the study must be documented clearly, and must be completed for any subject who has received any amount of drug during the treatment period. If the reason for early withdrawal is an AE or an abnormal laboratory value, the specific event or test result must also be recorded.

8.3 Procedures for Subjects Who Withdraw Early

Following early termination, the subject should be informed about which evaluations are necessary to monitor his or her safety. In addition, subjects should be encouraged to complete any procedures or evaluations outlined in Section 5.4, Study Termination/Early Study Termination.

8.4 Replacement of Subjects

Subjects who have not received TH-302, or withdraw consent prior to any assessment of disease response (clinical or radiographic), will be replaced. Subject enrollment numbers are unique and will not be re-assigned.

9. CONDITIONS FOR INITIATING, MODIFYING, OR TERMINATING THE STUDY

9.1 Institution Review

The investigator will submit this protocol, any protocol modifications, and the subject consent form to be used in this study to the local Institutional Review Board (IRB) for review and approval. A letter confirming IRB approval of the protocol and subject consent form, and an IRB approved informed consent form must be forwarded to CTRC prior to the enrollment of subjects into the study.

9.2 Informed Consent

The investigator or his or her designee must explain to the subject, in the presence of a witness, the purpose and nature of the study, the study procedures, and the possible adverse effects, and all other elements of consent as defined in 21 CFR Part 50 and Clinical Trial Directive or ICH E6 guidelines before enrolling that subject in the study. It is the investigator's (or designee's) responsibility to obtain informed written consent

from each subject, or if appropriate, the subject's parent or legal guardian.

9.3 Modifications

If any modifications in the experimental design, dosages, parameters, subject selection, or any other sections of the protocol are indicated or required, the investigator will consult with Threshold before such changes are instituted. Modifications will be accomplished through formal amendments and approval from the appropriate IRB.

9.4 Deviations

The PI will consider any deviations from the protocol on a case-by-case basis. The investigator or other designated alternate in his absence will contact the local DSMB (DSMB2) as soon as possible to discuss the associated circumstances. The principal investigator and the DSMB will then decide whether the subject should continue to participate in the study. All protocol deviations and the reasons for such deviations must be noted in the source documents and will be reported to the IRB as per standard institutional policy.

9.5 Termination

Although there are no predefined criteria for termination, if Threshold or the investigators) discover conditions during the course of the study that indicate it should be discontinued, an appropriate procedure for terminating the study will be instituted, including notification of the appropriate regulatory agencies and IRB.

10. INVESTIGATOR'S RESPONSIBILITIES

10.1 Responsibilities/Performance

The investigator will ensure that this study is conducted in accordance with all regulations governing the protection of human subjects. The investigator will adhere to the basic principles of "Good Clinical Practice," as outlined in Title 21 of the Code of Federal Regulations (CFR), Part 312, Subpart D, "Responsibilities of Sponsors and

Investigators"; 2 1 CFR, Part 50, "Protection of Human Subjects"; 21 CFR, Part 56, "Institutional Review Boards"; and the US Food and Drug Administration (FDA) guideline entitled "Good Clinical Practice: Consolidated Guideline". For studies conducted outside of the USA, the investigator will ensure adherence to the principles outlined in the International Conference on Harmonisation (ICH) E6 "Guideline for Good Clinical Practice". Additionally, this study will be conducted in compliance with the Declaration of Helsinki and with any local laws and regulations of the country in which the research is conducted. The investigator will ensure that all work and services described in or associated with this protocol will be conducted in accordance with the investigational plan, applicable regulations, and the highest standards of medical and clinical research practice. The investigator is responsible for the control of drugs under investigation. The investigator will provide copies of the study protocol and Investigator's Brochure to all sub- investigators, pharmacists, and other staff responsible for study conduct.

10.2 Confidentiality

The investigator must ensure that each subject's anonymity will be maintained and each subject's identity will be protected from unauthorized parties. A number will be assigned to each subject upon study entry and the number and the subject's initials will be used to identify the subject for the duration of the study. Documents submitted to CTRC should not identify a subject by name. Documents that are not submitted to CTRC (e.g, signed consent form) will be maintained by the investigator in strict confidence.

10.3 Informed Consent and Permission To Use Protected Health Information

It is the responsibility of the investigator to obtain written informed consent from each subject participating in this study after adequate explanation, in lay language, of the methods, objectives, anticipated benefits, and potential hazards of the study. The investigator must also explain that the subject is completely free to refuse to enter the study or to discontinue participation at any time (for any reason) and receive alternative conventional therapy as indicated. Prior to study participation, each subject will sign an IRB approved informed consent form, which will be in form and substance acceptable to Threshold, and receive a copy of same (and information leaflet, if appropriate). For subjects not qualified or able to give legal consent, consent must be obtained from a parent, legal guardian, or custodian.

The investigator or designee must explain to the subject before enrollment into the study that for evaluation of study results, the subject's protected health information obtained during the study may be shared with Threshold, regulatory agencies, and IECs/IRBs. It is the investigator's (or designee's) responsibility to obtain permission to use protected health information per HIPAA from each subject, or if appropriate, the subjects' parent or legal guardian

10.4 SOURCE DOCUMENTATION AND INVESTIGATOR FILES

The investigator must maintain adequate and accurate records to fully document the conduct of the study and to ensure that study data can be subsequently verified. These documents should be classified into 2 separate categories: (1) investigator study file and (2) subject clinical source documents that corroborate data collected for case report forms.

Subject clinical source documents would include hospital clinic patient records; physician's and nurse's notes; appointment book; original laboratory, ECG, EEG, radiology, pathology, and special assessment reports; pharmacy dispensing records; subject diaries; signed informed consent forms; consultant letters; and subject screening and enrollment logs.

The following will be documented in source documents at the site:

1) Medical history/physical condition and diagnosis of the subject before involvement in the study sufficient to verify protocol entry criteria (if not already present);
2) Study number , assigned subject number, and verification that written informed consent was obtained (each recorded in dated and signed notes on the day of entry into the study);
3) Progress notes for each subject visit (each dated and signed);

4) Study drug dispensing and return;
5) Review of laboratory test results;
6) Adverse events (action taken and resolution);
7) Concomitant medications (including start and stop dates); and
8) Condition of subject upon completion of or early termination from the study.

10.4.1 Exclusion Log

The investigator must keep a record listing all patients considered for entry into the study but subsequently excluded. The reason for each exclusion will be recorded in the Subject Exclusion Log.

11 DATA SAFETY MONTIORING PLAN

DataandSafetyMonitoringOversight

A Data and Safety Monitoring Plan is required for all an individual protocols conducted at CTRC. All protocols conducted at CTRC are covered under the auspices of the CTRC Institutional Data Safety Monitoring Plan (DSMP).

The CTRC Institutional DSMP global policies provide individual trials with:

- institutional policies and procedures for institutional data safety and monitoring,
- an institutional guide to follow,
- monitoring of protocol accrual by the CTRC Protocol Review Committee,
- review of study forms and orders by the Forms Committee,
- independent monitoring and source data verification by the CTRC QA Monitor/Auditor;
- tools for monitoring safety events,
- monitoring of UPIRSO's by the Director of Quality Assurance and DSMC,
- determining level of risk (Priority of Audit Level Score – PALS),
- oversight by the Data Safety Monitoring Committee (DSMC), and
- verification of protocol adherence via annual audit for all Investigator Initiated Studies by the CTRC Quality Assurance Division.

11.1 Monitoring Progress and Safety

Due to the risks associated with participation in this protocol, the CTRC DSMB2 in conjunction with the Principal Investigator will perform assessment of adverse events, adverse event trends and treatment effects on this study. The CTRC DSMB2 acts as an independent Data Safety Monitoring Board (DSMB) for IIS conducted at CTRC. The CTRC DSMB2 will monitor data throughout the duration of a study to determine if

continuation of the study is appropriate scientifically and ethically. An additional layer of review is provided by the CTRC Data Safety Monitoring Committee (DSMC) who will review the DSMB's reports.

Baseline events and adverse events will be captured using the CTRC Master Adverse Events Document for each patient using CTCAE V4.03 for the grading and attribution of adverse events. Usage of the CTRC Master Adverse Events Document centrally documents:

- the event and grades the seriousness of the event,
- if the event was a change from baseline,
- the determination of the relationship between the event and study intervention,
- if the event was part of the normal disease process, and
- what actions were taken as a result of the event.

Safety Definitions

For this study, the following safety definitions will be applicable:

AdverseEvent

An adverse event (AE) is defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research For this study, all adverse events will be documented starting after the first dose of study drug and ending 30 days after the last dose of study drug is received.

SeriousAdverseEvent

Any adverse event that:

1. results in death;
2. is life-threatening (places the subject at immediate risk of death from the event as it occurred);
3. results in inpatient hospitalization or prolongation of existing hospitalization;
4. results in a persistent or significant disability/incapacity;
5. results in a congenital anomaly/birth defect; or
6. based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition

UnanticipatedProblemsInvolvingRisksto SubjectsorOthersDefinition(**UPIRSO**):

Unanticipated problem involving risk to subjects or others includes any incident, experience or outcome that meets all of the following criteria:

A. unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied (note: the unfounded classification of a serious adverse event as "anticipated" constitutes serious non-compliance);

B. definitely related or probably related to participation in the research; and

C. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

11.2 Reporting Requirements

For this study, the Master Adverse Events Documents collected on patients for this protocol will be reviewed by the Principal Investigator and discussed with the Dana Farber team on a monthly basis to determine if a serious safety problem has emerged that results in a change or early termination of a protocol such as:

- dose modification,
- suspending enrollment due to safety or efficacy, or
- termination of the study due to a significant change in risks or benefits.

The PI will provide the DSMB2 with the quarterly findings for discussion and review during their quarterly meetings.

Specific areas of concern that will be reported to the DSMB2 regarding this study that would qualify as an endpoint are:

- evidence from interim evaluation suggesting TH302 would have no therapeutic benefit to patients on study
- unacceptable toxicity that would prevent patients from receiving treatment and therefore benefit

As per the CTRC DSMP, any protocol modifications, problematic safety reports, unanticipated problems, and suspension or early termination of a trial must be reported to the DSMB2 and all members of the research team. Furthermore, the PI of this study will promptly notify all study affiliates, the UTHSCSA IRB, the CTRC DSMC, and the FDA via a FDA Form 3500A written IND safety report of any adverse events that are either serious and/or unexpected.

UnanticipatedProblemInvolvingRiskToSubjectsOrOthers (**UPIRSO**) is defined as: Any incident, experience or outcome that meets all of the following criteria:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-

approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied
- definitely related or probably related to participation in the research; and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Suspension and early termination of a trial must also be reported immediately to the Director of Quality Assurance who will promptly notify the CTRC DSMC, FDA, all participating study sites, and the UTHSCSA IRB.

The PI will review the Master Adverse Events documents to determine the significance of the reported events and will provide findings using the Investigator Initiated Study Quarterly DSMC Report Form on a quarterly basis with the DSMB2. The DSMB2 will review the information provided by the PI and report to the CTRC DSMC on a quarterly basis unless an emergent issue has been identified. The Investigator Initiated Study Quarterly DSMC Report Form includes information on adverse events, current dose levels, number of patients enrolled, significant toxicities per the protocol, patient status (morbidity and mortality) dose adjustments with observed response, and any interim findings. Any trend consisting of three or more of the same event will be reported to the CTRC DSMB for independent review outside of the quarterly reporting cycle, which begins three months after the first subject is enrolled. The DSMB2 will also provide its findings to the CTRC's Regulatory Affairs Division so that it may be provided to the UTHSCSA IRB with the protocol's annual progress report. Conflict of interest is avoided by the independent reviews of the CTRC DSMB2, CTRC DSMC and by ongoing independent review of UPIRSO's by the Director of Quality Assurance.

All SAE and UPRISO's will be reported following CTRC, UTHSCSA institutional and FDA guidelines.

UTHSCSA SAE/UPIRSO REPORTING REQUIREMENTS **For IIS that the PI holds the IND**		
Type Event	Report to	Timeframe
All AE, SAE and UPIRSO	Regulatory Affairs and DQA	ASAP
SAE	PI at UTHSCSA	Within 24 hours
SAE	IRB	Annually
UPIRSO - all	PI at UTHSCSA	Within 24 hours
UPIRSO - all	FDA	Within 7 days
UPIRSO - life threatening	UTHSCSA IRB/UTHSCSA OCR	Within 48 hours

UPIRSO - non-life threatening	UTHSCSA IRB/UTHSCSA OCR	Within 7 days

11.3 Assuring Compliance with Protocol and Data Accuracy

As with all studies conducted at CTRC, the PI has ultimate responsibility for ensuring protocol compliance, data accuracy/integrity and responding to recommendations that emanate from monitoring activities. Protocol compliance, data accuracy and reporting of events is further ensured by an annual audit conducted by the Data Safety Officer, whose audit report is shared with the PI, the research team and will be reviewed by the CTRC DSMC.

CTRCDSMBMembership

The CTRC has two DSMB's with a primary set of members specific to the histology of the study consisting of UTHSCSA faculty and staff. This Protocol will utilize DSMB#2.
As per NCI guidelines and to eliminate conflict of interest (financial, intellectual, professional, or regulatory in nature), the CTRC DSMB specific to this study will not treat patients on this protocol. Usage of the DSMB specific to the histology has been created to ensure that experts in that histology are represented on the DSMB assembled for this protocol, but may be expanded, at the PI's discretion, to include other members which may include:

- Experts in the fields of medicine and science that are applicable to the study (if not currently represented on the DSMB),
- Statistical experts,
- Lay representatives,
- Multidisciplinary representation, from relevant specialties including experts such as bioethicists, biostatisticians and basic scientists, and
- Others who can offer an unbiased assessment of the study progress.

Additional or alternate membership of in the DSMB is selected by the DSMC chair, in conjunction with the PI of this protocol.

CTRCDSMBCharterandResponsibilities

The CTRC DSMB will provide information on the membership composition, including qualifications and experience to both the UTHSCSA IRB and CTRC PRC for review. The CTRC DSMB for this study will act as an independent advisory board to the PI and will report its findings and recommendations to the PI, the UTHSCSA IRB and the CTRC DSMC. CTRC DSMB reports will utilize the Investigator Initiated Study DSMC Report Form Once the protocol is activated, if not already established elsewhere in the protocol the CTRC DSMB will establish and provide:

- procedures for maintaining confidentiality;
- statistical procedures including monitoring guidelines, which will be used to monitor the identified primary, secondary, and safety outcome variables;

- consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study;
- plans for changing frequency of interim analysis as well as procedures for recommending protocol changes;
- recommendation of dose escalation, MTD recommendation of early termination based on efficacy results;
- recommendation of termination due to unfavorable benefit-to-risk or inability to answer study questions;
- recommendation of continuation of ongoing studies;
- recommend modification of sample sizes based on ongoing assessment of event rates; and
- review of final results and publications.

11.4 –Multi-Site Data Monitoring, Requirements and Expectations

In order to assure timely review of data for safety and quality of data, CTRC requires participating institutions to submit participant source documents for review. Data is expected to be updated in a timely manner to facilitate review.

On-site and/or remote monitoring of case report forms will occur after the first subject is enrolled and treated on the protocol. Additional monitoring may occur at the request of CTRC. Participating institutions are required to provide access to source documents. All source documents will be submitted to CTRC with the subject's HIPAA information deidentified and only including the patient's protocol subject ID number assigned at the time of patient registration.

All data submitted to CTRC will be monitored for timeliness, accuracy, compliance with protocol and state/federal requirements. Results from monitoring and audits will be

shared with any participating sites and the CTRC PI in a timely manner. Any required corrections or responses to audit findings are expected to be received back to the CTRC QA Division within 30 days.

CTRC, as the coordinating site, will distribute any protocol amendments and IND safety reports to the participating institution's submission to their local IRB. Participating institutions are expected to submit and maintain local IRB approval. Copies of the participating site's IRB approval letter, consent documents and any subsequent amendments are required to be on file with CTRC prior to consent of a subject.

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Appendix A: Schedule of Assessments

Study Procedures	Screening (w/in 21 days of C1D1)	Cycle 1			Cycle 2			Cycle 3			Cycle 4+						EOT[G]
Day		Day 1	Day 15	Day 29	Day 1	Day 15	Day 29	Day 1	Day 15	Day 29	Day 1	Day 15	Day 29				
Informed Consent	X																
Demographics	X																
Med/Surg History	X																
Physical Exam (to include Adverse events and conc meds)[F,H]	X	X[A]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]			X[B]						X
Vital signs	X	X[A]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]				X
Height	X	X[A]															
Weight	X	X[A]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]				X
ECOG	X	X[A]	X[B]	X[B]	X[B]	X[B]		X[B]			X[B]						X
ECG	X	X[k]	X[k]														
Hematology labs	X	X[A]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]				X
Chemistry labs	X	X[A]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]				X
Coagulation labs[C]	X	X[A]															X
Urinalysis[D]	X	X[A]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]				X
Pregnancy test	X	X[A]						X[B]									X
TH-302[F]		X	X	X	X	X	X	X	X	X	X	X	X				
Bevacizumab[F]		X	X	X	X	X	X	X	X	X	X	X	X				
MDASI-BT Questionairre	X				X[j]			X[j]			X[j]						X
Tumor Assessment[E]	X	X[A,E]			X[B,E]			X[B,E]			X[B,E]						
Serum/Whole Blood Sampling for Biomarkers[H]		X[A]			X[B]			X[B]			X[B]						X
^{18}F-FMISO-PET		X[B, L]			X[B,I, L]												

Footnotes for Appendix A

A: May be done up to 5 days prior to visit.

B: May be done up to 3 days prior to visit.

C: INR only if pt is on warfarin

D: Screen & EOT: UA with micro. Other visits, urine dipstick for protein & glucose. If positive or change from BL, complete urinalysis w/ micro.

E: Tumor Assessments: At screening & after every cycle (within 3 days).

F: Plus or minus 2 days.

G: Occurs 3-4 weeks after the last dose of TH-302 for subjects who terminate early.

H: Testing includes hypoxia biomarkers (GLUT-1, HIF-1a, LDH-A, VEGF) as well as angiogenesis/vasculogenesis biomarkers(SDF1, bFGF1, circulating endothelial cells/ progenitor circulating cells). Done pre-dose on Day 1.

I: Optional assessments

J: Done prior to dosing

K: Triplicate ECG done within 30 minutes prior to TH-302 infusion within 30 minutes after the end of TH-302 infusion

L: To include plasma pharmacokinetic collection to assess decay of 18F-FMISO

APPENDIX B: EASTERN COOPERATIVE ONCOLOGY GROUP (ECOG) PERFORMANCE SCORE SCALE

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead.

APPENDIX C: RANO TUMOR RESPONSE

Table: Summary of the RANO Response Criteria

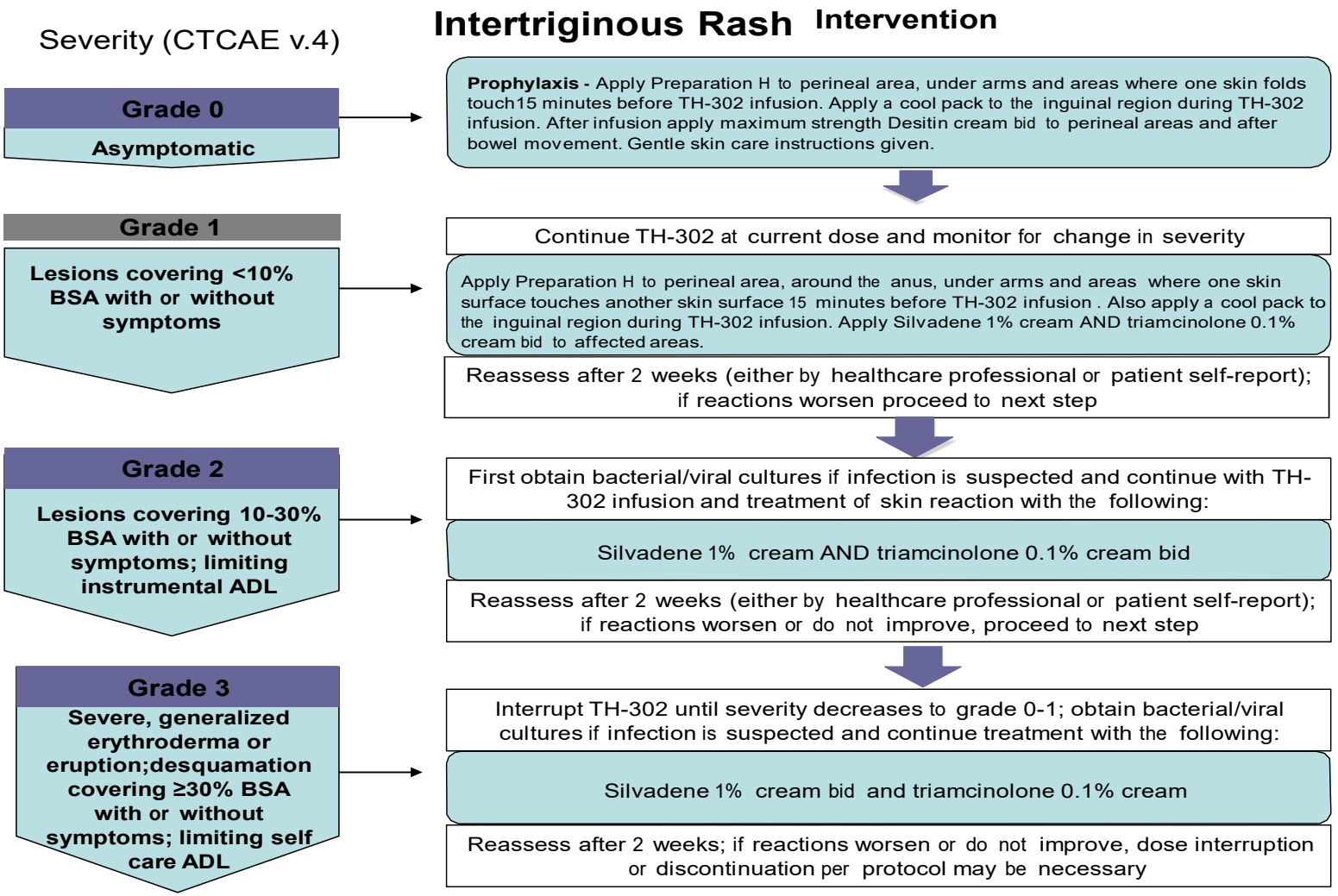
	CR	PR	SD	PD#
T1-Gd +	None	≥50% decrease	<50% decrease-<25% increase	≥25% increase*
T2/FLAIR	Stable or decrease	Stable or decrease	Stable or decrease	Increase*
New Lesion	None	None	None	Present*
Corticosteroids	None	Stable or decrease	Stable or decrease	**NA**
Clinical Status	Stable or increase	Stable or increase	Stable or increase	Decrease*
Requirement for Response	All	All	All	Any*

CR=complete response; PR=partial response; SD=stable disease; PD=progressive disease

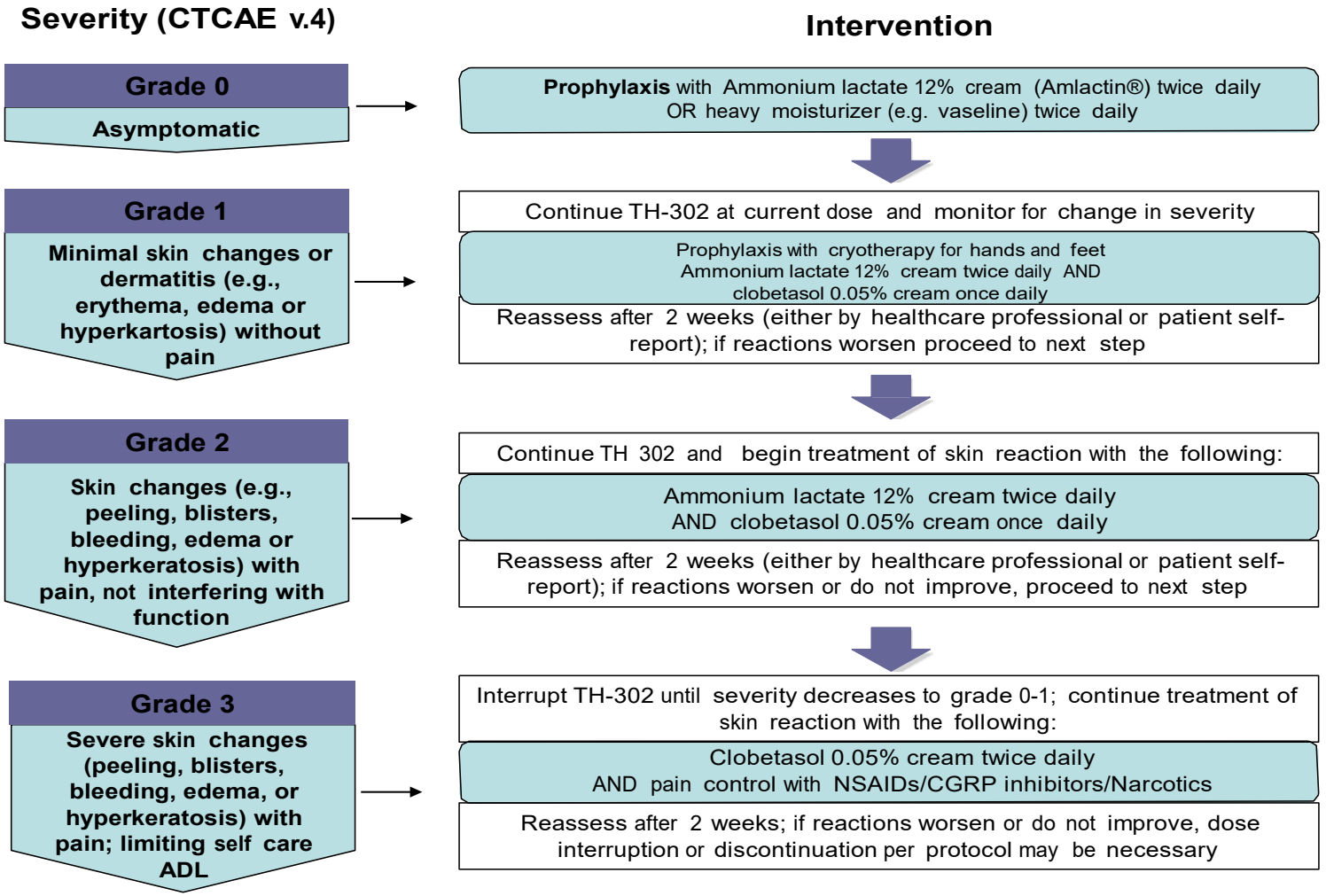
Progression occurs when any of the criteria with * is present

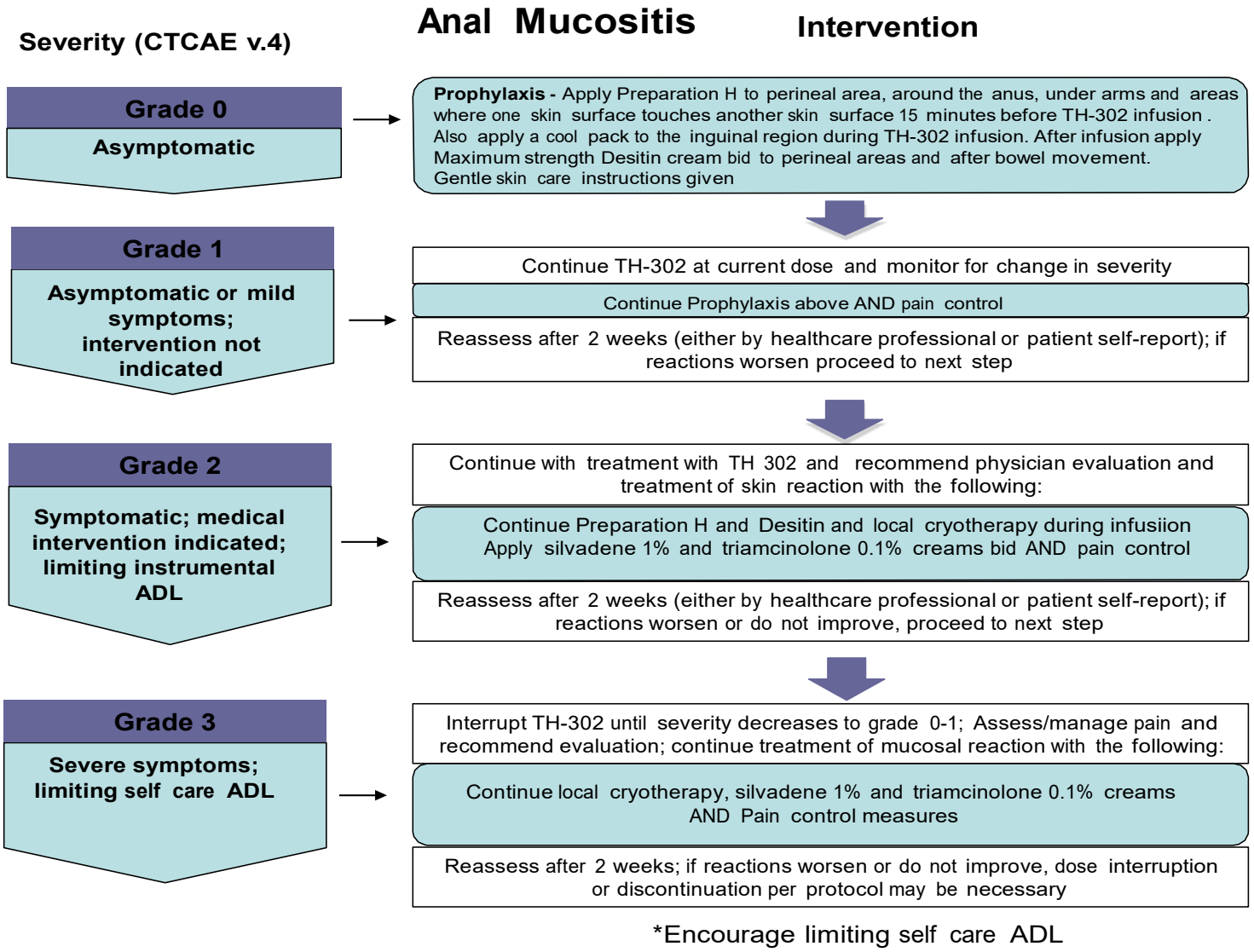
NA: Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration

Appendix D: TH-302 Skin and Mucosal Toxicity Treatment Algorithms

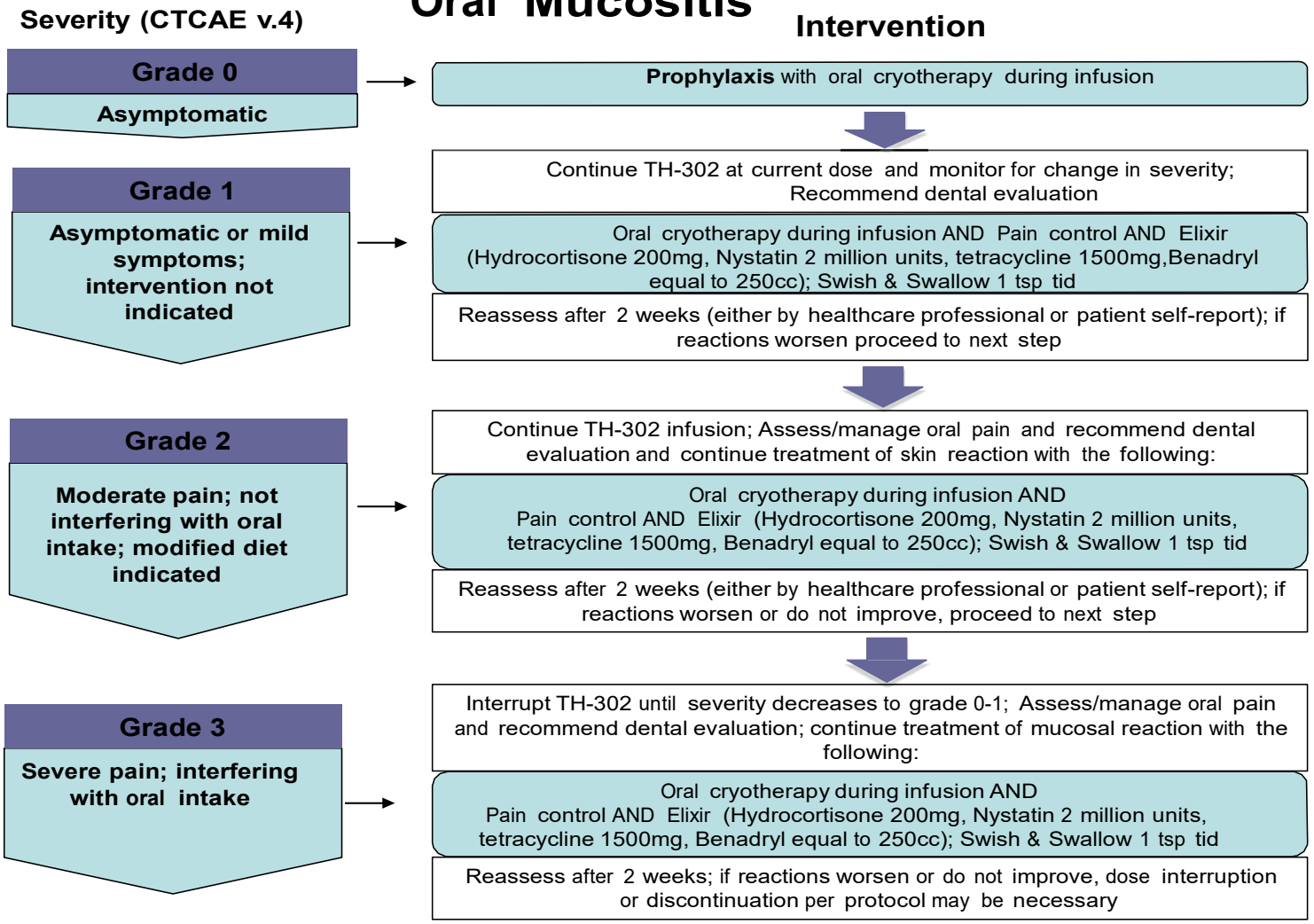


Hand-Foot Skin Reaction



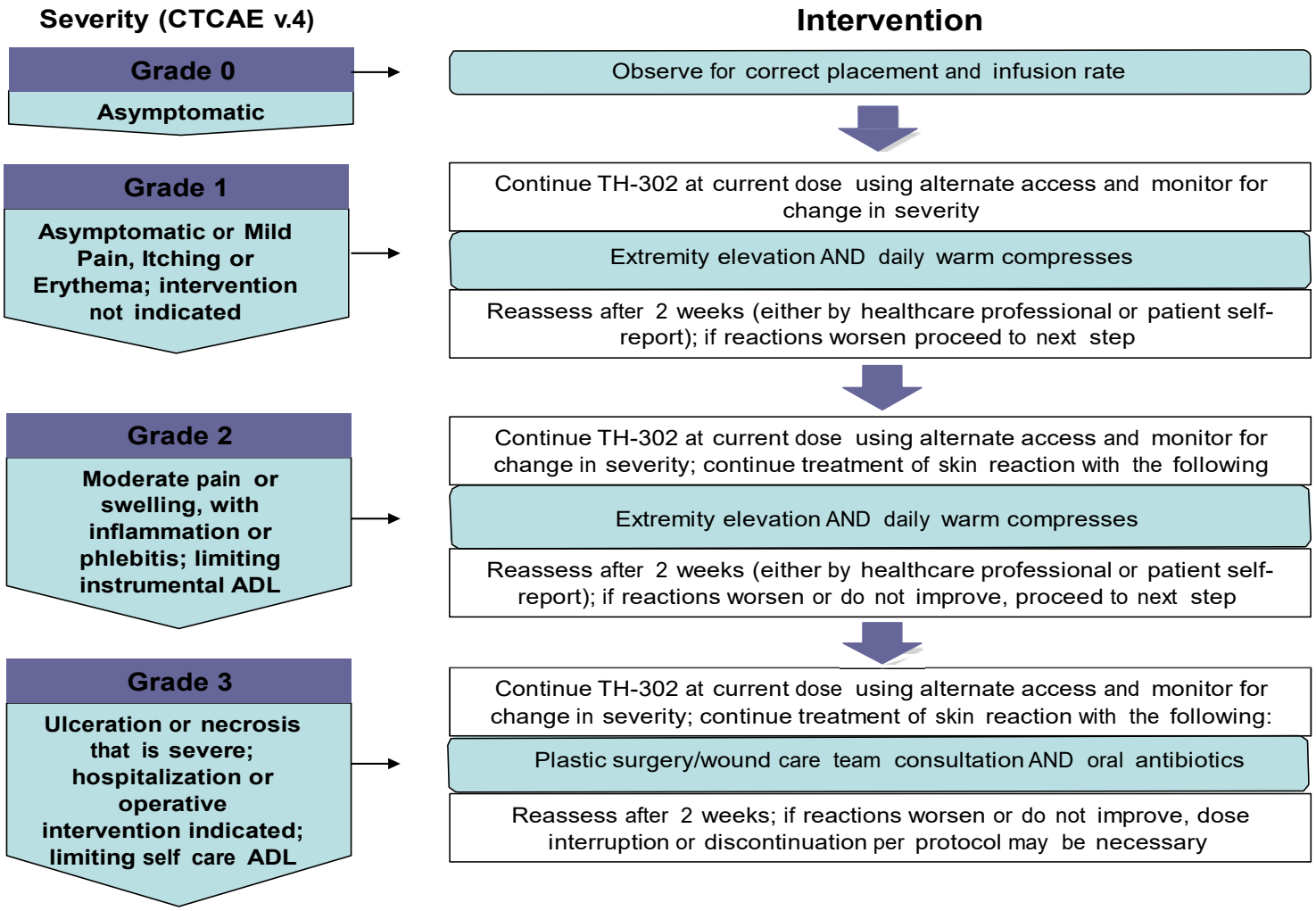

Anal Mucositis
Severity (CTCAE v.4)
Intervention
Grade 0
Asymptomatic
Prophylaxis - Apply Preparation H to perineal area, around the anus, under arms and areas where one skin surface touches another skin surface 15 minutes before TH-302 infusion . Also apply a cool pack to the inguinal region during TH-302 infusion. After infusion apply Maximum strength Desitin cream bid to perineal areas and after bowel movement. Gentle skin care instructions given
Grade 1
Asymptomatic or mild symptoms; intervention not indicated
Continue TH-302 at current dose and monitor for change in severity
Continue Prophylaxis above AND pain control
Reassess after 2 weeks (either by healthcare professional or patient self-report); if reactions worsen proceed to next step
Grade 2
Symptomatic; medical intervention indicated; limiting instrumental ADL
Continue with treatment with TH 302 and recommend physician evaluation and treatment of skin reaction with the following:
Continue Preparation H and Desitin and local cryotherapy during infusiion
Apply silvadene 1% and triamcinolone 0.1% creams bid AND pain control
Reassess after 2 weeks (either by healthcare professional or patient self-report); if reactions worsen or do not improve, proceed to next step
Grade 3
Severe symptoms; limiting self care ADL
Interrupt TH-302 until severity decreases to grade 0-1; Assess/manage pain and recommend evaluation; continue treatment of mucosal reaction with the following:
Continue local cryotherapy, silvadene 1% and triamcinolone 0.1% creams AND Pain control measures
Reassess after 2 weeks; if reactions worsen or do not improve, dose interruption or discontinuation per protocol may be necessary
*Encourage limiting self care ADL

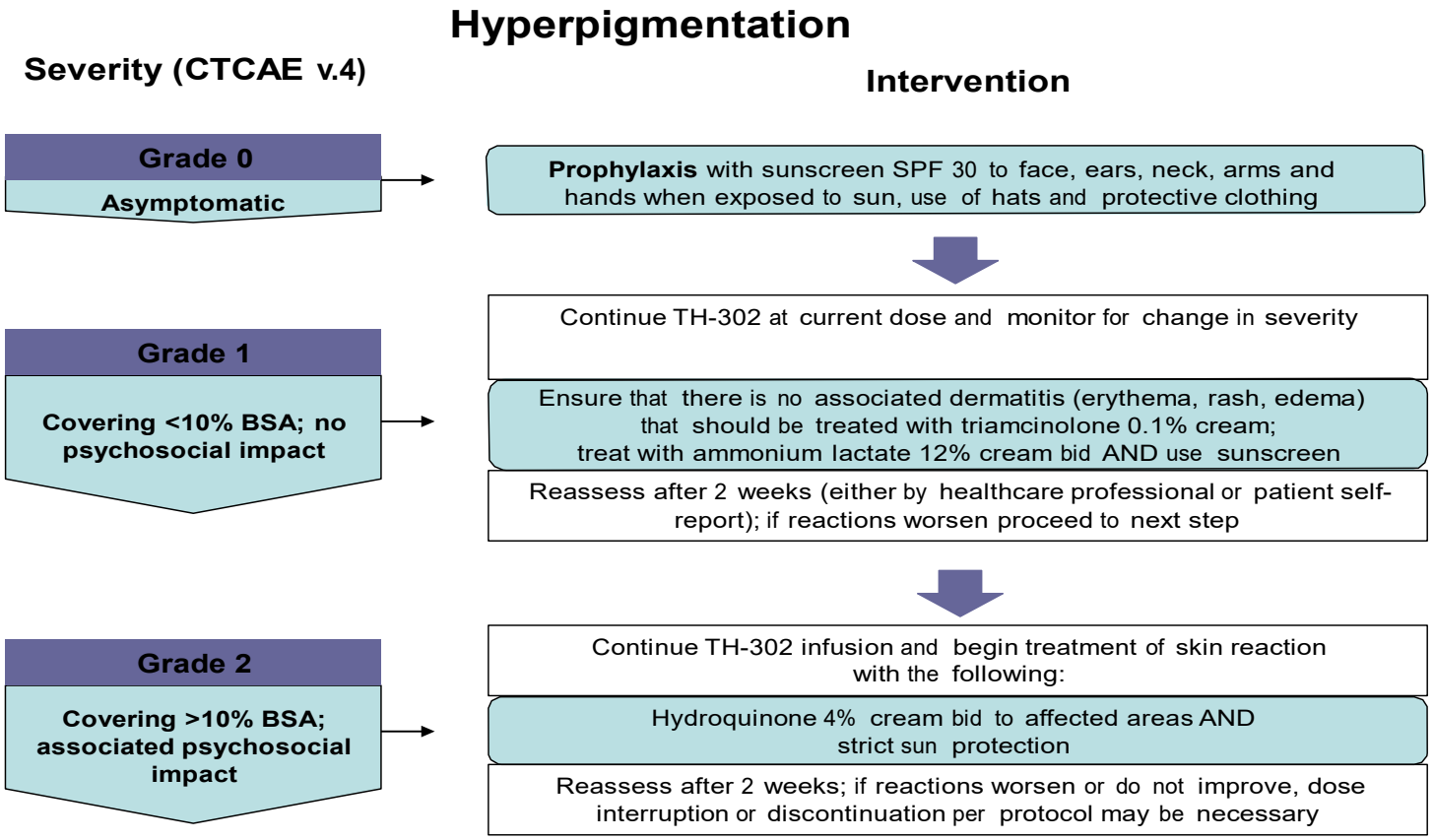
Oral Mucositis



*Encourage use of soft toothbrush

Injection site reaction




Hyperpigmentation
Severity (CTCAE v.4)
Intervention
Grade 0
Asymptomatic
Prophylaxis with sunscreen SPF 30 to face, ears, neck, arms and hands when exposed to sun, use of hats and protective clothing
Grade 1
Covering <10% BSA; no psychosocial impact
Continue TH-302 at current dose and monitor for change in severity
Ensure that there is no associated dermatitis (erythema, rash, edema) that should be treated with triamcinolone 0.1% cream; treat with ammonium lactate 12% cream bid AND use sunscreen
Reassess after 2 weeks (either by healthcare professional or patient self-report); if reactions worsen proceed to next step
Grade 2
Covering >10% BSA; associated psychosocial impact
Continue TH-302 infusion and begin treatment of skin reaction with the following:
Hydroquinone 4% cream bid to affected areas AND strict sun protection
Reassess after 2 weeks; if reactions worsen or do not improve, dose interruption or discontinuation per protocol may be necessary

Appendix E: MD. Anderson Symptom Inventory-Brain Tumor (1\IDASI-BT)

Date: r;:Qt QJ/

Subjec t Jnlda ls:o::lJ

Study Name: ——————————

Prototol#: ——————————

P : ——————————

MD Anderson # | | | | | | |

PDMS # | | |

M. D. Anderson Symptom Inventory - Brain Tumor (MDASI - Bli)

Part I. How sewre are)'0\W symptoms?

People with cancer frequent y have symptoms that are caused by their disease or by their treatment. We ask you to rate how severe the following symptoms l'lave been In the last 24 hours. Pleue fl0 in the circl e below from 0 (symptom has not been present) to 10 (the symptom was as bad as you can Imagine H could be) for each Item.

	Not Present 0	1	2	3	4	5	6	7	8	9	As Bad As You Can Imagine 10
1. Your pain at its WORST?	○	○	○	○	○	○	○	○	○	○	○
2. Your fatigue (tiredness) at its WORST?	○	○	○	○	○	○	○	○	○	○	○
3. Your nausea at its WORST?	○	○	○	○	○	○	○	○	○	○	○
4. Your disturbed sleep at its WORST?	○	○	○	○	○	○	○	○	○	○	○
5. Your feeling of being distressed (upset) at its WORST?	○	○	○	○	○	○	○	○	○	○	○
6. Your shortness of breath at its WORST?	○	○	○	○	○	○	○	○	○	○	○
7. Your problem with remembering things at its WORST?	○	○	○	○	○	○	○	○	○	○	○
8. Your problem with lack of appetite at its WORST?	○	○	○	○	○	○	○	○	○	○	○
9. Your feeling drowsy (sleepy) at its WORST?	○	○	○	○	○	○	○	○	○	○	○
10. Your having a dry mouth at its WORST?	○	○	○	○	○	○	○	○	○	○	○
11. Your feeling sad at its WORST?	○	○	○	○	○	○	○	○	○	○	○
12. Your vomiting at its WORST?	○	○	○	○	○	○	○	○	○	○	○
13. Your numbness or tingling at its WORST?	○	○	○	○	○	○	○	○	○	○	○
14. Your weakness on one side of the body at its WORST?	○	○	○	○	○	○	○	○	○	○	○
15. Your difficulty understanding at its WORST?	○	○	○	○	○	○	○	○	○	○	○
16. Your difficulty speaking (finding the words) at its WORST?	○	○	○	○	○	○	○	○	○	○	○

SAMPLE

Caw: unthl/ ,W

Subjtet nltlols:

MD Anderson# | | | | | | | POMS # | | |

Study Name:
Protocol:
PI:

	Not Present 0	1	2	3	4	5	6	7	8	9	As Bad As Yo Can Imagine 10
17. Your seizures at its WORST?	○	○	○	○	○	○	○	○	○	○	O
18. Your difficulty **concentrating** at its WORST?	○	○	○	○	○	○	○	○	○	○	O
19. Your vision at its WORST?	○	○	○	○	○	○	○	○	○	○	○
20. Your change in **appearance** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
21. Your change in bowel pattern (diarrhea or constipation) at its WORST?	○	○	○	○	○	○	○	○	○	○	○
22. Your **irritability** at its WORST?	○	○	○	○	○	○	○	○	○	○	○

SAMPLE

Port M.How havo yo.. symptoms **iptoms interfered ...th ... li...**

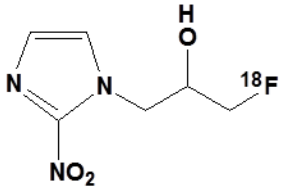
Symptom& froquently intarfere with **fere with ... we ... function.** How much have your oymptoms Intarforod **with the following items in the last 2...**

	Did not interfere 0	1	2	3	4	5	6	7	8	9	Interfered Completely 10
General activity?	○	○	○	○	○	○	○	○	○	○	○
2•. Mood?	O	O	O	O	O	O	O	O	O	O	O
25. Work (including work around the house)?	○	○	○	○	○	○	○	○	○	○	○
26. Relations with other people?	○	○	○	○	○	○	○	○	○	○	○
27 —	○	○	○	○	○	○	○	○	○	○	○
28. Enjoymonl lifo?	○	○	○	○	○	○	○	○	○	○	○

APPENDIX F: PET IMAGING PROCEDURES

TRACER:

Molecular Characterization:



Storage Conditions: 20 mL Sterile Syringe at room temperature.

Handling Procedures: According to established Institutional guidelines for safe handling of radioactive material

Dilution: Diluted with normal saline to an activity of 3.7 MBq/kg (0.1 mCi/kg) (maximum 260 MBq, 7 mCi) with a final volume of < 15 mL.

Dosimetry Assessment: gamma-ray dose calibrator as described

PET Scan Acquisition and Reconstruction

All PET imaging will be performed on a research-dedicated Siemens/CTI HR+. This PET system acquires 63 simultaneous axial (horizontal) planes over a transaxial field of view of 15.5 cm, sufficient to cover the entire brain. The in-plane resolution is 4.1 mm FWHM (full-width at half maximum), equal to that used in the fMRI studies. Emission scans will be obtained for FMISO (see details in the following paragraphs). Transmission scans will be performed with a rotating 10 mCi pin source. Emission images will be reconstructed using a standard filtered back-projection algorithm with a 6 mm FWHM high-resolution Hahn filter, including attenuation correction computed from the transmission scans. Reconstructed images are transferred to an image analysis workstation for further processing in MATLAB 7.

BF Computational Strategy

Hypoxia imaging using ^{18}FMISO. Patients will not be required to fast. Venous access lines will be established in each arm, one for FMISO injection and the other for blood sampling. A transmission scan will be obtained for 15 min with the patient in the supine position. Patients will be injected intravenously with 3.7 MBq/kg (0.1 mCi/kg) of ^{18}FMISO, maximum 370 MBq (10 mCi). Images will be acquired in three dimensional mode with 63 slices covering a 15.5-cm axial field of view. A single field of view emission scan (30 min) of the brain with tumor will be obtained approximately 120 min post-injection. During emission tomography, four venous blood samples will be collected at intervals of 5 min. Whole blood samples of 1 mL each will be counted in a calibrated well counter. ^{18}FMISO blood activity will be averaged and then expressed as mCi/mL (or MBq/ml) of decay corrected to time of injection. Reconstructed ^{18}FMISO PET images will be scaled to the average venous blood concentration of ^{18}FMISO activity to produce tumor-to-blood (T/B) values. A three dimensional pixel by pixel T/B calculation will be performed. The number of pixels in the brain with a T/B ratio of ≥1.2, indicating viable hypoxic tissue, will be determined and converted to cubic millimeters to assess hypoxic volume (HV). This cut-off value of 1.2 has been used to determine HV in previous studies exploring ^{18}FMISO PET[2-6]. Various T/B ratios will be used to determine HV in order to confirm and verify that a T/B ratio of 1.2 is reasonable to determine HV.

References for Appendix F

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