SUMMARY OF CHANGES

For Protocol Amendment

NCI Protocol #: 9552
Local Protocol #: 14-079

NCI Version Date: TBD
Protocol v9.0 Date: 04-October-2016

#	Section	Page(s)	Change
1.	Title page	3	Protocol version date change to: 04-october-2016
2.	7.2.4	54-61	Update CAEPR list v2.4 dated 5/23/16 for Bevacizumab

NCI Protocol #: 9552
Local Protocol #: 14-079

TITLE: A Phase 1 study of MLN0128 and bevacizumab in patients with recurrent glioblastoma and other solid tumors

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NCI-Supplied Agent: MLN0128 (Millennium Pharmaceuticals, Inc.)
Other Agent(s): Bevacizumab (commercial)
IND #:
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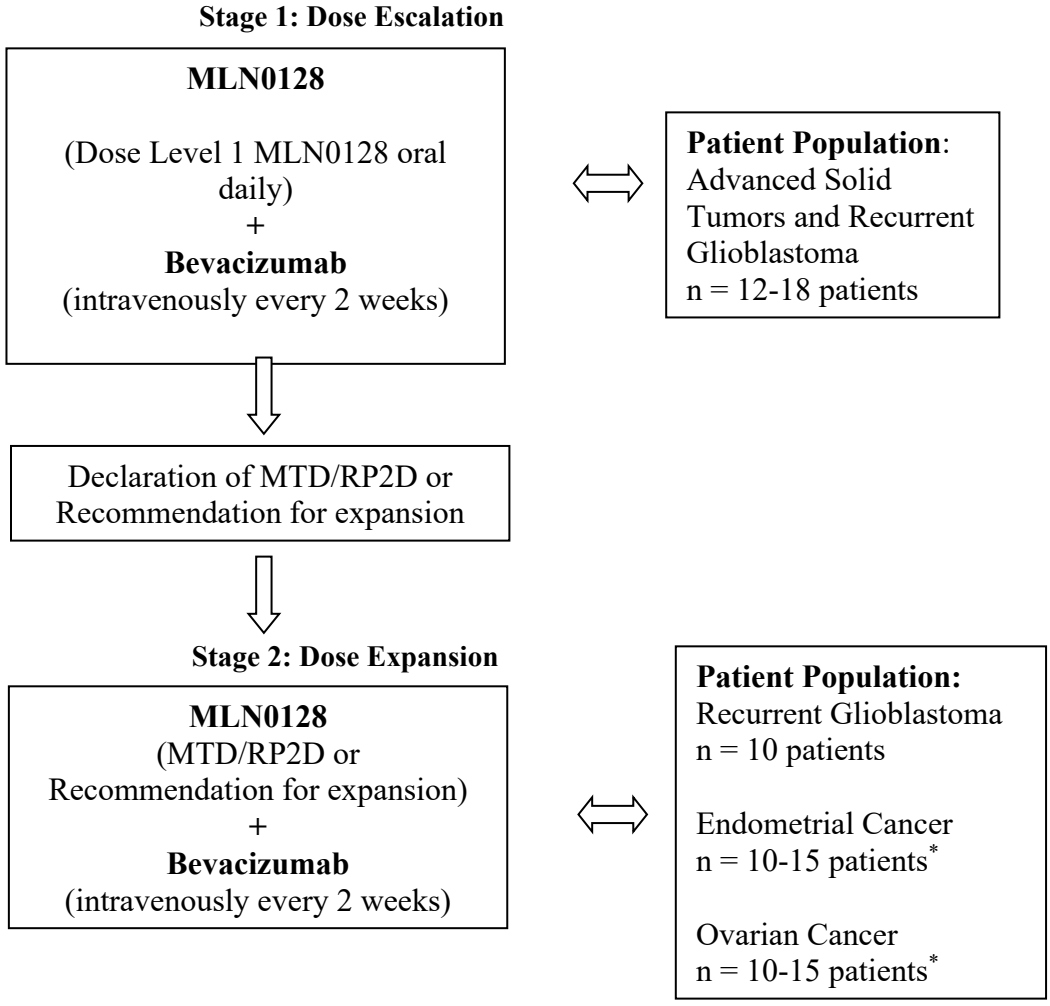
SCHEMA

This study will involve 2 stages.



Stage 1: **Dose escalation to estimate MTD/RP2D**.
Approximately 12-18 patients with advanced solid tumors including recurrent glioblastoma (GBM) will be enrolled using a standard 3+3 design, including 3-6 patients in each cohort. Patients will start at dose level 1, 3mg daily of MLN0128, based on the recommended single agent daily dose of MLN0128 by Millennium. MTD for the current study will be defined as the highest dose at which <2/6 patients experience DLT. Bevacizumab will be administered at 10mg/kg IV every 2 weeks. Should 2/6 patients at the initial dose level experience DLT, then 3 patients will be enrolled at dose level -1 (MLN0128 3mg orally 5 out of 7 days with standard dose bevacizumab.)

Stage 2: Expansion cohort at MTD.
After the MTD/RP2D is determined, stage 2 will enroll and treat up to an additional 40 patients (10-15* patients with endometrial cancer, 10-15* patients with ovarian cancer and 10 patients with recurrent GBM) to: 1)better characterize the safety profile of MLN0128; 2) evaluate plasma and CSF concentrations of MLN0128 when administered with and without bevacizumab; and 3) evaluate pharmacodynamic properties of MLN0128 in tumor tissue and plasma. Patients in stage 2 will receive MLN0128 daily starting with cycle 1 day 1 and bevacizumab 10mg/kg IV every 2 weeks starting with cycle 1 day 15. Bevacizumab will not be administered cycle 1 day 1.

*may enroll up to 15 participants in these two dose expansion cohorts in order to obtain sufficient biopsy material from 10 patients per cohort for correlative study requirements

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1. OBJECTIVES

1.1 Primary Objectives

- To determine the maximum tolerated dose and recommended phase 2 dose (MTD/RP2D) of daily oral MLN0128 when administered with bevacizumab in patients with advanced solid tumors including recurrent glioblastoma (GBM).
- To evaluate the overall safety and tolerability of the combination of MLN0128 and bevacizumab.

1.2 Secondary Objectives

- To assess the preliminary anti-tumor activity of the combination of MLN0128 and bevacizumab, as determined by response rate (RR), progression-free survival (PFS) and overall survival (OS).
- To assess tolerability throughout study therapy with MLN0128 and bevacizumab, including beyond the MTD interval with the following measures of cumulative treatment-related toxicities:
    - Frequency of toxicities leading to missed doses or delays
    - Percentage of cycles given or not within 7 days of their scheduled times
    - Percentage of actual planned dosage administration
    - Percentage of patients that discontinue study drugs due to treatment related toxicity.

1.3 Exploratory Objectives

- To assess CSF penetration of MLN0128 in combination with bevacizumab in patients with recurrent GBM by evaluating the plasma and CSF concentrations of MLN0128 in the absence and presence of bevacizumab.
- To perform archival tumor analysis for markers of dysregulated cell signaling that may predict response to mTOR inhibitor therapy such as EGFR (expression by IHC and amplification by FISH), PTEN (expression by IHC and deletion by FISH), p-AKT, p-S6K, p-4EBP, p-mTOR and p-Erk in patients with recurrent GBM.
- To analyze select phosphorylated proteins (ERK, AKT, mTOR, 4EBP1, GSK3beta, p70S6K, rS6) from tumor biopsies obtained at baseline and after treatment with MLN0128 from endometrial and ovarian cancer patients enrolled in stage 2.
- To analyze circulating plasma levels of angiogenic growth factors before, during and after treatment with MLN0128 and bevacizumab
  To perform genetic mutation analysis and proteomic analysis of tissue from biopsies of endometrial and ovarian cancer patients including analysis of KRAS, BRAF, PIK3CA, AKT1 and PTEN.

2. BACKGROUND

2.1 Study Diseases

2.1.1 Glioblastoma

Glioblastoma (GBM) is the most common type of malignant primary brain tumor with over 10,000 new cases diagnosed each year in the United States[1]. Despite optimal treatment with surgery, radiation therapy and temozolomide, most patients have tumor recurrence and the median survival is only approximately 15 months[2]. Following disease progression, most chemotherapeutic agents have minimal activity[1]. Bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor (VEGF) has shown modest activity, producing a 26% response rate and a median survival of approximately 9 months from recurrence [3,4].

In the past decade, there has been important progress in understanding the molecular pathogenesis of GBM [5,6]. The phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR signaling axis plays a central role in cell growth, survival, motility, and metabolism [7] in a variety of cancers including GBM[5]. Data from TCGA suggests that over 80% of GBM patients have activation of this pathway as a result of either PIK3Ca or PIK3R1 mutations, inactivation of PTEN, or amplification or mutation of receptor tyrosine kinases such as EGFR, PDGFR and MET [5]. Inhibition of this pathway represents an attractive therapeutic strategy. Prior clinical trials evaluating mTOR inhibitors have generated modest evidence of anti-tumor activity although assessment of effective penetration of these agents through the blood brain barrier was not carefully determined in the prior trials[8,9].

2.1.2 Endometrial Cancer

Endometrial cancer is diagnosed in approximately 40,000 women in the US every year, and long term outcomes for patients with advanced or recurrent disease are poor[10]. New therapeutic approaches and combinations are needed to improve outcomes in this patient population. There are limited treatment options with in the advanced disease setting. Inactivation of the PTEN (phosphatase with tensin homology) tumor-suppressor gene is the most common genetic defect in endometrial cancers, seen in up to 80% of endometrioid (type 1) tumors[11]. PIK3CA activating mutation are also seen in 36% of endometrial type 1 carcinomas, most frequently in those tumors that also bear PTEN mutation[12]. When PTEN is deleted, mutated or otherwise inactivated, activation of PI3K (phosphatidylinositol 3-kinase) effectors, particularly AKT and the downstream mTOR can occur in the absence of any exogenous stimulus

Given the strong data supporting dysregulation of the PI3K/AKT/mTOR pathway, the rapalogs have generated significant interest in endometrial cancer. A single-agent, multi-institution trial of temsirolimus in patients with recurrent and/or metastatic endometrial cancer were recently reported[13]. The study had two investigational arms:

Group A was chemotherapy naïve and Group B had been exposed to one prior line of chemotherapy. Four (14%) of 29 evaluable patients in Group A had a partial response, and 20 (69%) had a stable disease; the median PFS was 7.33 months for the group. Of 25 evaluable patients in Group B, 1 (4%) patient had a partial response and 12 (48%) had stable disease. Median PFS was 3.25 months in Group B. Stable disease required a minimum of 4 weeks duration. Treatment was better tolerated in the chemotherapy naïve group; however sixteen of sixty patients in the combined arms discontinued therapy due to toxicity (26.6%). The combination of bevacizumab and temsirolimus was investigated in a phase 2 study for endometrial cancer patients having previously received 1-2 lines of chemotherapy and/or radiation. This study reported a 24.5% response rate including one complete response and a 6-month progression-free survival (PFS6) of 46.9%. The combination in this pretreated population was also associated with significant toxicities, including gastrointestinal-vaginal fistulas, intestinal perforations, epistaxis and thromboembolism[14].

2.1.3 Ovarian Cancer

There is an unmet need to develop effective and novel treatment strategies for epithelial ovarian cancer (EOC). It is the leading cause of death from gynecologic malignancies in the United States[10]. Targeted molecular therapies against the cancer cell and surrounding stromal cells such as fibroblasts, endothelial cells, and inflammatory cells, are being developed as the signaling pathways that drive tumor development and progression are elucidated. EOCs induce extensive neovascularization that facilitates tumor growth and metastasis. Hollingsworth et al. demonstrated that increased vasculature correlates with higher grade EOC tumors and poor survival[15]. Multiple therapies have been developed to target the interactions between tumor cells and the stroma, including anti-angiogenic agents, exemplified by bevacizumab, a monoclonal antibody against vascular endothelial growth factor (VEGF) that has some single agent phase II clinical activity in platinum-resistant EOC[16]. However, this and other single biologic agents have not extended the progression-free or overall survival in EOC remarkably. Combination of bevacizumab with other targeted agents has significantly increased its efficacy in early phase 1 and phase 2 trials in EOC.

Approximately 40% of ovarian cancers have increased AKT2 activity[17,18]. Downstream of AKT, phosphorylated (*p*)mTOR and *p*GSK-3β correlate with AKT activation status in > 80% of ovarian cancers, validating activation of the PI3K/AKT pathway [17]. Thus, pharmacologic agents that inhibit downstream targets of the PI3K/AKT signaling pathway such as mTOR might preferentially kill tumor cells having AKT dependence for survival not shared by normal cells[19]. A phase 2 trial of temsirolimus in recurrent EOC demonstrated a 9% response rate and a 24% PFS at six months indicating modest activity of this TORC1 inhibitor[20].

2.2 CTEP IND Study Agent: MLN 0128

The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR)

signaling axis plays a central role in cell growth, survival, motility, and metabolism in a variety of cancers[21,22]. Several studies have examined the role of mTOR inhibitors, namely, rapamycin and its analogs (rapalogs) for the treatment of cancer including sirolimus, temsirolimus, everolimus, and ridaforolimus[9,23-25]. However, these agents have achieved limited efficacy for several reasons. First, all of these inhibitors selectively inhibit TORC1 but fail to block TORC2, and therefore may result in activation of Akt *via* the S6K-IRS1 feedback loop and via mTORC2-mediated phosphorylation of AKT at serine 473 (Ser473)[26]. Additionally, TORC1 inhibition by rapalogs may lead to activation of the MAPK pathway[27]. In the brain, many of the rapalogs also have only limited penetration across the blood-brain barrier (BBB) and may fail to achieve effective intra-tumoral therapeutic concentrations.

The TOR kinase-domain inhibitors of mTOR (TORKinibs) differ in their mode of action from rapalogs by binding to the ATP-binding site of the kinase domain of mTOR, thus resulting in inhibition of both mTOR complexes, TORC1 and TORC2. Inhibition of TORC2 has been shown to reverse the AKT activation induced by TORC1 inhibition[28].

MLN0128 is a potent, orally available ATP-competitive inhibitor of both TORC1 and TORC2 complexes. It has been shown to inhibit phosphorylation of TORC1 downstream targets, including S6 and 4EBP1. Additionally, it inhibits phosphorylation of AKT, a TORC2 target a variety of tumor cell lines, including HER2-amplified trastuzumab-sensitive and –resistant breast cancer cells, breast ductal T47D cells that harbor PI3KCA mutation, as well as metastatic renal cell cancer (RCC) and endometrial cell lines[22,29-31].

Summaries of Nonclinical and Clinical Studies

The pharmacodynamics and antitumor activity of MLN0128 was studied *in vivo* in murine xenograft models of human glioblastoma, non-small cell lung cancer (NSCLC), breast cancer, renal cell cancer, endometrial adenocarcinoma, and castration-resistant prostate cancer (CPRC). Consistent with the mode of action, MLN0128 inhibited phosphorylation of downstream modulators of mTORC1 (namely 4EBP1 and S6) and mTORC2 (namely AKT [S473]) in human U87 glioblastoma tumor xenograft models in mice at doses as low as 0.1 mg/kg. Additionally, MLN0128 showed strong tumor growth inhibition (TGI) in all 8 xenograft models at tolerable oral (PO) doses from 0.15 mg/kg (daily [QD]; tested in MDA-MB-361 breast carcinoma) to 3.0 mg/kg (every other day [Q2D] or once weekly [QW]; tested in all models).

Single-agent MLN0128 is in clinical development in two phase 1 studies in subjects with advanced solid malignancies and hematologic malignancies (multiple myeloma [MM] and Waldenstrom macroglobulinemia [WM]), and in a third study in combination with paclitaxel with or without trastuzumab in subjects with advanced solid tumors.

Study INK128-001

Study INK128-001 is evaluating safety and anti-tumor activity of MLN0128 in subjects with advanced solid malignancies. As of 09 December 2012, 106 subjects have been treated in Study INK128-001. The most common adverse events (AEs) (≥ 20%), regardless of causality were hyperglycemia (64%), nausea (60%), vomiting (49%), decreased appetite (40%), diarrhea (37%), asthenia (35%), fatigue and mucosal inflammation (28% each), rash (27%), and pruritus (24%). Most commonly reported (> 3%) Grade ≥ 3 AEs, regardless of causality, include hyperglycemia

(10%), asthenia (7%), anemia (6%), lymphopenia (6%), hypophosphatemia (5%), mucosal inflammation (5%), and rash (5%).

In Study INK128-001, as of 09 December 2012, the maximum tolerated doses (MTD) for all 4 schedules has been determined: for the QD dosing the MTD is 6 mg QD, for the QDx3dQW dosing, the MTD is 16 mg; for the QDx5dQW dosing, the MTD is 10 mg; and for the QW dosing schedule, the MTD is 40 mg. The MTDs for each of the 4 schedules was determined by evaluation of cohorts of 6 evaluable patients. At each MTD, up to 6 additional patients were enrolled to further evaluate safety and tolerability. A significant proportion of patients treated at the MTDs required dose modifications due to drug-related AEs beyond 1 or 2 cycles, and therefore were not representative of a recommended phase 2 dose. The study is currently further evaluating doses at less than the MTD for QDx3dQW and QDx5dQW, to determine a dose(s) and schedule(s) to be studied further in the expansion phase of the study, as well as in future phase 2 studies. The dose escalation portion of the study has evaluated dose regimens ranging from 2 to 7 mg QD, 7 to 40 mg QW, 6 to 20 mg QDx3dQW, and 7 to 13 mg QDx5dQW.

Study INK128-002
Study INK128-002 is evaluating safety and anti-tumor activity of MLN0128 in subjects with hematologic malignancies (MM and WM). As of 09 December 2012, 39 subjects have been treated in Study INK128-002. The most common AEs (≥ 20%), regardless of causality were fatigue and nausea (51% each), hyperglycemia (38%), thrombocytopenia (36%), diarrhea (26%), decreased appetite and vomiting (23% each), and stomatitis/anemia (21% each). Most commonly reported (at least 2 subjects) Grade ≥ 3 AEs, regardless of causality included thrombocytopenia (18%), fatigue (10%), neutropenia (8%), hypocalcemia (5%), hypophosphatemia (5%), mucosal inflammation (5%), and pneumonia (5%).

In Study INK128-002, dose escalation is completed, with 4 mg determined as the MTD for the QD schedule, and 9 mg determined as the MTD for the QDx3dQW schedule.

Study INK128-003
Study INK128-003 is evaluating safety and anti-tumor activity of MLN0128 in subjects with advanced solid tumors in combination with paclitaxel (and trastuzumab for HER2+ subjects). As of 09 December 2012, 48 subjects have been treated in Study INK128-003; no subject has been treated with trastuzumab. The most common AEs (≥ 20%), regardless of causality were fatigue (67%); nausea (56%); diarrhea (50%); dehydration and hyperglycemia (44% each); anemia (40%); anorexia, mucosal inflammation, and vomiting (38% each); rash (35%); asthenia and neutropenia (31% each); hypokalemia (27%); hypophosphatemia and urinary tract infection (23% each); and constipation (21%). Most commonly reported (at least 2 subjects) Grade ≥ 3 AEs, regardless of causality, include neutropenia (23%); hypophosphatemia (17%); diarrhea, fatigue, and hyperglycemia (15%); and dehydration (10%).

In the dose expansion phase of this study, additional subjects were enrolled once the MTD was determined and evaluated for each of the dosing schedules. These subjects were enrolled into an arm of HER2- subjects receiving MLN0128 in combination with paclitaxel (n = 11) at the MTD or an arm of HER2+ subjects receiving MLN0128 in combination with paclitaxel plus weekly trastuzumab (n = 2) at the MTD. The most common AEs (≥ 20%), regardless of causality, were

alopecia, fatigue, and nausea (23% each) and anorexia, asthenia, diarrhea, dyspepsia, mucosal inflammation, neutropenia, and vomiting (15% each). Most commonly reported (at least 2 subjects) Grade ≥ 3 AEs in the dose expansion phase of INK128-003, regardless of causality, include mucosal inflammation, neutropenia, and pneumonia (reported by 1 subject each in the HER2- arm).

In Study INK128-003, dose escalation is completed, with 8 mg of MLN0128 QDx3dQW being selected for the dose expansion phase of the study. The QDx5dQW and QW schedules were abandoned before MTDs were declared, as these schedules were viewed as less convenient relative to the QDx3dQW schedule, from the perspective of administering the paclitaxel and trastuzumab combination. The dose expansion portion of this study is ongoing, with HER2-/unknown patients receiving 8 mg of MLN0128 QDx3dQW in combination with paclitaxel, and HER2+ patients receiving 8 mg of MLN0128 QDx3dQW in combination with paclitaxel and trastuzumab.

Nonclinical and Clinical Pharmacokinetics

MLN0128 is rapidly absorbed after oral administration to mice, rats, dogs, and monkeys, with high oral bioavailability.

A study of the tissue distribution of [^{14}C]MLN0128 showed that [^{14}C]MLN0128 was rapidly and widely distributed throughout the body in Long-Evans rats; radioactivity was eliminated from most tissues at 48 hours postdose, and from all but the adrenal cortex, adrenal gland, adrenal medulla, eye, liver, and uveal tract at 168 hours. MLN0128 displayed dose proportional plasma exposures and a moderate propensity to cross the blood-brain barrier. MLN0128 was modestly bound to human plasma proteins (approximately 70%). MLN0128 inhibited breast cancer resistance protein (BCRP), organic cation transporter (OCT)1, and OCT2.

M1, the single metabolite (monohydroxylation product) observed in human microsomal incubations, was also observed in rats and monkeys, the species used for the Good Laboratory Practice (GLP) toxicology studies. The main isozymes responsible for phase 1 metabolism appear to be cytochrome P450 (CYP) 2C9, 2C19, and 3A4. MLN0128 displayed low potential (IC50 > 30 μM) for inhibition of CYP1A2, 2C19, 2C8, 2C9, CYP2D6, and 3A4. MLN0128 did not induce CYP1A2, 2B6, and 3A4 activity and expression at concentrations up to 30 μM.

Biliary and urinary excretion of MLN0128 and its monohydroxylation metabolite (M1) was investigated in bile duct-cannulated male Sprague-Dawley rats over 24 hours after administration of a single IV dose of 0.5-mg/kg MLN0128(38) The biliary elimination of MLN0128 and M1 each represented less than 1% of the total MLN0128 dose. The urinary excretion of MLN0128 and M1 represented less than 1% and 21%, respectively, of the total MLN0128 dose.

Oral administration of MLN0128 in humans has a low potential for metabolic and transporter-based drug-drug interactions (DDIs), especially given clinical exposures observed to date after administration of the highest single dose (total maximum plasma concentration [C_{max}] of 0.64 μM [free C_{max} of 0.19 μM] at 40 mg QW).

MLN0128 does not inhibit P-glycoprotein (P-gp).

Preliminary pharmacokinetic (PK) data from phase 1 studies in humans indicate that MLN0128 exhibits fast oral absorption (first time to maximum plasma concentration [T_{max}] generally between 1 to 4 hours after dosing) and dose-linear pharmacokinetics with a mean plasma half-life of ~8 hours and does not accumulate meaningfully in plasma on either dosing regimen. The pharmacokinetics of MLN0128 was generally consistent with no appreciable differences across the three phase 1 studies. Neither paclitaxel nor MLN0128 appeared to alter the PK of the other agent when co-administered.

2.2.1 **Bevacizumab (rhuMAb VEGF)**

Bevacizumab is a humanized IgG1 monoclonal antibody (MAb) that binds all biologically active isoforms of human VEGF (or VEGF-A) with high affinity (kd = 1.1 nM). The antibody consists of a human IgG1 framework and the antigen-binding complementarity-determining regions from the murine anti-VEGF MAb A.4.6.1.16-18. Bevacizumab is commercially available and FDA approved as monotherapy for patients with recurrent glioblastoma. It is currently approved in combination therapy for certain solid tumors, such as advanced non-squamous non-small cell lung cancer, metastatic renal cell cancer, and metastatic colorectal cancer.

Mechanism of Action

VEGF is one of the most potent and specific angiogenic factors, and it has been identified as a crucial regulator of both normal and pathological angiogenesis. VEGF is a secreted, heparin-binding protein that exists in multiple isoforms. Action of VEGF is primarily mediated through binding to the receptor tyrosine kinases VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1). The biologic effects of VEGF include endothelial cell mitogenesis and migration, increased vascular permeability, induction of proteinases leading to remodeling of the extracellular matrix, and suppression of dendritic cell maturation. Neutralization of VEGF by A.4.6.1 or bevacizumab has been shown to inhibit the VEGF-induced proliferation of human endothelial cells in vitro and to decrease microvessel density and interstitial pressure in tumor xenografts in vivo.

Preclinical Studies

The murine parent MAb of bevacizumab, A4.6.1, has demonstrated potent growth inhibition in vivo in a variety of human cancer xenograft and metastasis models, including those for SKLMS-1 leiomyosarcoma, G55 glioblastoma multiforme, A673 rhabdomyosarcoma, Calu-6, and MCF-7 cell lines [32-34]. The antitumor activity was enhanced with the combination of A4.6.1 and chemotherapeutic agents compared to either agent alone. Furthermore, combined blockage of the VEGF pathway and other growth factor pathways (e.g., EGFR or PDGFR) has also demonstrated additive effects in vivo [35,36]. Associated with the antitumor activity of anti-VEGF MAbs were findings of reduced intratumoral endothelial cells and microcapillary counts as well as reduced vascular permeability and interstitial pressure.

Nonclinical toxicology studies have examined the effects of bevacizumab on female reproductive function, fetal development, and wound healing. Fertility may be impaired in Cynomolgus monkeys administered bevacizumab, which led to reduced uterine weight and

endometrial proliferation as well as a decrease in ovarian weight and number of corpora lutea. Bevacizumab is teratogenic in rabbits, with increased frequency of fetal resorption as well as specific gross and skeletal fetal alterations. In juvenile Cynomolgus monkeys with open growth plates, bevacizumab induced epiphyseal dysplasia that was partially reversible upon cessation of therapy. Bevacizumab also delays the rate of wound healing in rabbits, and this effect appeared to be dose dependent and characterized by a reduction of wound tensile strength.

Clinical Studies

Bevacizumab has been studied in multiple Phase I, Phase II, and Phase III clinical trials and in multiple tumor types. The following discussion summarizes bevacizumab's safety profile and presents some of the efficacy results pertinent to this particular trial. Clinical proof of principle for anti-VEGF therapy with bevacizumab has been provided by the pivotal Phase III trial of bevacizumab (5 mg/kg every 2 weeks) in combination with bolus irinotecan/5-fluorouracil/leucovorin (IFL) in patients with untreated advanced colorectal cancer (AVF2107g) [37]. In that study, the addition of bevacizumab to IFL was associated with an increase in objective responses (45% vs. 35%) and significant prolongations of both time to progression (10.6 vs. 6.2 months) and overall survival (20.3 vs. 15.6 months) compared with IFL.

Based on the survival advantage, bevacizumab was approved in 2004 in the United States for first-line treatment in combination with IV 5-FU-based chemotherapy for subjects with metastatic colorectal cancer. Additional data from Phase III trials in metastatic CRC [38], and non-small cell lung cancer [39] have also demonstrated clinical benefit from bevacizumab when added to chemotherapy.

Single agent bevacizumab received accelerated FDA approval in 2009 based on favorable results from two Phase II clinical trials in recurrent GBM [3,4]. In these studies, PFS6 for bevacizumab monotherapy ranged from 29% to 42.6%. Compared to a historical PFS6 of 15% for recurrent GBM [40], these studies suggest that bevacizumab has significant clinical activity in this patient population.

There is evidence that angiogenesis also plays a role in endometrial cancer progression and prognosis[41]. Fifty-two patients with one or two rounds of prior systemic therapy were treated with single agent bevacizumab in the GOG study 229E[42]. Seven patients (13.5%) had clinical responses (one complete response and six partial responses, with six months median response duration) and 21 patients (40.4%) survived progression free for at least six months. Median PFS and OS times were 4.2 and 10.5 months, respectively.

In ovarian cancer, single agent bevacizumab has shown some clinical activity, but no impact on OS[16,43]. However, its combination with other targeted agents has shown promise in early phase I and II trials[44,45].

Safety Profile in Humans

In the initial Phase I and II clinical trials, four potential bevacizumab-associated safety signals were identified: hypertension, proteinuria, thromboembolic events, and hemorrhage. Additional

completed Phase II and Phase III studies of bevacizumab as well as spontaneous reports have further defined the safety profile of this agent. Bevacizumab-associated adverse events identified in Phase III trials include congestive heart failure (CHF) primarily in metastatic breast cancer, gastrointestinal perforations, wound healing complications, and arterial thromboembolic events (ATE).

Hypertension: An increased incidence of hypertension has been observed in patients treated with bevacizumab. Grade 4 and 5 hypertensive events are rare. Clinical sequelae of hypertension are rare but have included hypertensive crisis, hypertensive encephalopathy, and reversible posterior leukoencephalopathy syndrome (RPLS) [37,46]. There is no information on the effect of bevacizumab in patients with uncontrolled hypertension at the time of initiating bevacizumab therapy. Therefore, caution should be exercised before initiating bevacizumab therapy in these patients. Monitoring of blood pressure is recommended during bevacizumab therapy. Optimal control of blood pressure according to standard public health guidelines is recommended for patients on treatment with or without bevacizumab. Temporary interruption of bevacizumab therapy is recommended in patients with hypertension requiring medical therapy until adequate control is achieved. If hypertension cannot be controlled with medical therapy, bevacizumab therapy should be permanently discontinued. Bevacizumab should be permanently discontinued in patients who develop hypertensive crisis or hypertensive encephalopathy.

Proteinuria: An increased incidence of proteinuria has been observed in patients treated with bevacizumab compared with control arm patients. In the bevacizumab-containing treatment arms of clinical trials (across all indications), the incidence of proteinuria (reported as an adverse event) was up to 38% (metastatic CRC Study AVF2192g) [47]. The severity of proteinuria has ranged from asymptomatic and transient events detected on routine dipstick urinalysis to nephrotic syndrome; the majority of proteinuria events have been Grade 1. NCI-CTC Grade 3 proteinuria was reported in up to 3% of bevacizumab-treated patients, and Grade 4 in up to 1.4% of bevacizumab-treated patients. The proteinuria seen in bevacizumab clinical trials was not associated with renal impairment and rarely required permanent discontinuation of bevacizumab therapy.

Bevacizumab should be discontinued in patients who develop Grade 4 proteinuria (nephrotic syndrome). Patients with a history of hypertension may be at increased risk for the development of proteinuria when treated with bevacizumab. There is evidence from the dose-finding, Phase II trials (AVF0780g, AVF0809s, and AVF0757g) suggesting that Grade 1 proteinuria may be related to bevacizumab dose. Proteinuria will be monitored by urine protein: creatinine (UPC) ratio at least every 4 weeks.

Thromboembolic Events: Both venous and arterial thromboembolic (TE) events, ranging in severity from catheter-associated phlebitis to fatal, have been reported in patients treated with bevacizumab in the colorectal cancer trials, the recurrent glioblastoma trial and, to a lesser extent, in patients treated with bevacizumab in NSCLC and breast cancer trials. Venous thromboembolic events (VTE) have also been observed in trials with bevacizumab and glioblastoma. To assess the overall risk of VTE associated with the use of bevacizumab, a systemic review and meta-analysis was performed and included prospective randomized controlled trials in which standard antineoplastic therapy was used with and without

bevacizumab [48]. A total of 7,956 patients with a variety of advanced solid tumors from 15 trials were indentified. Among the patients treated with bevacizumab, the rates of all-grade and high-grade VTE were 11.9% and 6.3, respectively. Patients treated with bevacizumab had a significantly increased risk of VTE compared with controls (RR 1.31). Since TE events are very common in GBM independent of treatment [2], the relationship of thromboembolism to bevacizumab in this population is uncertain. Based on a Phase II clinical trial of bevacizumab with or without irinotecan in recurrent GBM, the rates of arterial thromboembolism were 2.4%-2.5% and venous thromboembolism were 3.6%-8.9% [3]. The first incidence of VTE will therefore not constitute a DLT.

An increased incidence of arterial thromboembolic events (ATE) was observed in patients treated with bevacizumab compared with those receiving control treatment. ATE include cerebrovascular accidents, myocardial infarction, transient ischemic attacks (TIAs), and other ATE. The analysis of pooled data of 1,745 patients from five randomized trials using bevacizumab combined with chemotherapy showed an increased risk of ATE (3.8% in treatment arm vs. 1.7% in the control arm) but not VTE [49]. Most ATE episodes described were myocardial or cerebrovascular events. Development of an ATE event was associated with a prior ATE event or age ≤ 65 years.

Gastrointestinal perforation: Patients may be at increased risk for the development of gastrointestinal perforation and fistula when treated with bevacizumab and steroids or chemotherapy. Bevacizumab should be permanently discontinued in patients who develop gastrointestinal perforation. A causal association of intra-abdominal inflammatory processes and gastrointestinal perforation to bevacizumab treatment has not been established. Nevertheless, caution should be exercised when treating patients with intra-abdominal inflammatory processes with bevacizumab. A meta-analysis of 17 randomized controlled trials demonstrated a significantly increased risk of gastrointestinal perforation in patients treated with bevacizumab compared to control medication [50]. The incidence was 0.9%, and the risks varied with tumor type, with colorectal cancer and renal cell cancer having the highest risk. In a Phase II clinical trial of bevacizumab with or without irinotecan for patients with recurrent GBM, 2.1%-2.5% experienced a Grade 3 gastrointestinal perforation [3,4].

Fistula: Bevacizumab use has been associated with serious cases of fistulae including events resulting in death. Fistulae in the GI tract are common (1%–10% incidence) in patients with metastatic CRC, but uncommon (0.1%□1%) or rare (0.01%–0.1%) in other indications. In addition, fistulae that involve areas of the body other than the GI tract (e.g. tracheoesophageal, bronchopleural, urogenital, biliary) have been reported uncommonly (0.1%–1%) in patients receiving bevacizumab in clinical studies and postmarketing reports. Events were reported at various timepoints during treatment, ranging from 1 week to > 1 year following initiation of bevacizumab, with most events occurring within the first 6 months of therapy.

Wound healing complications: Wound healing complications such as wound dehiscence have been reported in patients receiving bevacizumab. Most clinical trials with bevacizumab have required at least 28 days from any major surgery before starting treatment [51]. In a retrospective analysis of randomized trials in metastatic colorectal cancer, for a subset of patients who had surgeries 28-60 days before initiating bevacizumab, the incidence of wound complications were

low (1.3%) [52], indicating that the 28-day interval from colonic surgery might be appropriate. However, in the subset of patients undergoing emergent surgery while on study, 13% of the patients in the bevacizumab arm developed Grade 3 or Grade 4 postoperative wound complications compared to 3.4% of the patients in the chemotherapy arm. Surgery in patients currently receiving bevacizumab is not recommended. No definitive data are available to define a safe interval after bevacizumab exposure with respect to wound healing risk in patients receiving elective surgery; however, the estimated half-life of bevacizumab is 21 days. Bevacizumab should be discontinued in patients with severe wound healing complications. If patients receiving treatment with bevacizumab require elective major surgery, it is recommended that bevacizumab be held for 4–8 weeks prior to the surgical procedure. Patients undergoing a major surgical procedure should not begin or restart bevacizumab until 4 weeks after that procedure (in the case of high-risk procedures such as liver resection, thoracotomy, or neurosurgery, it is recommended that bevacizumab be restarted no earlier than 4 weeks after surgery). In a Phase II clinical trial of bevacizumab with or without irinotecan in patients with recurrent GBM, Grade 3 or higher wound-healing complications were reported in 1.3-2.4% [3].

Hemorrhage: Overall, grade 3 and Grade 4 bleeding events were observed in 4.0% of 1132 patients treated with bevacizumab in a pooled database from eight Phase I, Phase II, and Phase III clinical trials in multiple tumor types [53]. The hemorrhagic events that have been observed in bevacizumab clinical studies were predominantly tumor-associated hemorrhage (See below: Tumor-Associated Hemorrhage) and minor mucocutaneous hemorrhage.

Tumor-Associated Hemorrhage: Major hemorrhage has been observed primarily in patients with NSCLC. Life-threatening and fatal hemoptysis was identified as a bevacizumab-related adverse event in NSCLC trials. These events occurred suddenly and presented as major or massive hemoptysis. GI hemorrhages, including rectal bleeding and melena have been reported in patients with CRC, and have been assessed as tumor associated hemorrhages. Grades 1-4 tumor-associated hemorrhages were only very rarely seen in patients with GBM (less than 4%) [3]. Two of the five patients who developed intracranial hemorrhage were anticoagulated at the time of the hemorrhage; both hemorrhages were Grade 1.

Mucocutaneous Hemorrhage: Across all bevacizumab clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of patients treated with bevacizumab [53]. These were most commonly NCICTC Grade 1 epistaxis that lasted less than 5 minutes, resolved without medical intervention and did not require any changes in bevacizumab treatment regimen. There have also been less common events of minor mucocutaneous hemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

Reversible Posterior Leukoencephalopathy Syndrome: There have been rare reports of bevacizumab-treated patients developing signs and symptoms that are consistent with RPLS, a rare neurologic disorder that can present with the following signs and symptoms (among others): seizures, headache, altered mental status, visual disturbance, or cortical blindness, with or without associated hypertension. Brain imaging is mandatory to confirm the diagnosis of RPLS. In patients who develop RPLS, treatment of specific symptoms, including control of hypertension, is recommended along with discontinuation of bevacizumab. The safety of reinitiating bevacizumab therapy in patients previously experiencing RPLS is not known. In a

Phase II clinical trial of bevacizumab with or without irinotecan in patients with recurrent GBM, only one patient (1.3%) experienced serious reversible posterior leukoencephalopathy syndrome [3].

Congestive heart failure: In clinical trials CHF was observed in all cancer indications studied to date, but predominantly in patients with metastatic breast cancer. In the Phase III clinical trial of metastatic breast cancer (AVF2119g), 7 (3%) bevacizumab-treated patients experienced CHF, compared with two (1%) control arm patients [54]. These events varied in severity from asymptomatic declines in left ventricular ejection fraction (LVEF) to symptomatic CHF requiring hospitalization and treatment. All the patients treated with bevacizumab were previously treated with anthracyclines (doxorubicin cumulative dose of 240□360 mg/m2). Many of these patients also had prior radiotherapy to the left chest wall. Most of these patients showed improved symptoms and/or left ventricular function following appropriate medical therapy [54]. No information is available on patients with preexisting CHF of New York Heart Association (NYHA) Class II-IV at the time of initiating bevacizumab therapy, as these patients were excluded from clinical trials.

Adverse events in GBM studies from FDA labeling information: Bevacizumab is commercially available and FDA approved for patients with recurrent glioblastoma. For further details, see the bevacizumab FDA labeling information available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/125085s0169lbl.pdf.

All adverse events were collected in 163 patients enrolled in a non-comparative Phase II study who either received Bevacizumab alone or Bevacizumab plus irinotecan [3]. All patients received prior radiotherapy and temozolomide. Bevacizumab was administered at 10 mg/kg every 2 weeks alone or in combination with irinotecan. Bevacizumab was discontinued due to adverse events in 4.8% of patients treated with Bevacizumab alone.

In patients receiving Bevacizumab alone (N=84), the most frequently reported adverse events of any grade were infection (55%), fatigue (45%), headache (37%), hypertension (30%), epistaxis (19%) and diarrhea (21%). Of these, the incidence of Grade ≥ 3 adverse events was infection (10%), fatigue (4%), headache (4%), hypertension (8%) and diarrhea (1%). Two deaths on study were possibly related to Bevacizumab: one retroperitoneal hemorrhage and one neutropenic infection.

In patients receiving Bevacizumab alone or Bevacizumab plus irinotecan (N=163), the incidence of Bevacizumab-related adverse events (Grade 1−4) were bleeding/hemorrhage (40%), epistaxis (26%), CNS hemorrhage (5%), hypertension (32%), venous thromboembolic event (8%), arterial thromboembolic event (6%), wound-healing complications (6%), proteinuria (4%), gastrointestinal perforation (2%), and RPLS (1%). The incidence of Grade 3−5 events in these 163 patients were bleeding/hemorrhage (2%), CNS hemorrhage (1%), hypertension (5%), venous thromboembolic event (7%), arterial thromboembolic event (3%), wound-healing complications (3%), proteinuria (1%), and gastrointestinal perforation (2%).

2.3 Other Agent(s)

Not Applicable

2.4 Rationale

MLN0128 was demonstrated to be efficacious in five human tumor cell-line xenograft mouse models, including glioblastoma (GBM) U87MG (mutant PTEN), non-small cell lung cancer (NSCLC) A549 (mutant KRAS/LKB-1), breast carcinoma ZR-75-1 (mutant PTEN), RCC 786-O (mutant PTEN/VHL), and endometrial adenocarcinoma AN3-CA (mutant PTEN), demonstrating strong inhibition of tumor growth in these models compared to controls (untreated animals). Depending on the tumor type, tumor growth inhibition ranged from 32%-57% at 0.3 mg/kg QD and 60%-94% at 1 mg/kg QD. Tumor inhibition achieved at 3 mg/kg QOD (45%-99%) was similar to that obtained at 1 mg/kg QD in all five xenograft models. In U87MG GBM xenografts, MLN0128 produced dose-dependent inhibition of phosphorylation of 4EBP1, S6K and AKT [22]. Preclinical data have indicated that MLN0128 displays a low to moderate propensity to cross the BBB; steady-state plasma concentrations exceed brain concentrations by 5-fold in mice receiving a single 10-mg/kg oral dose of MLN0128[22].

In a phase I dose escalation study of MLN0128 (INK128) given orally daily in patients with advanced solid tumors, the maximum tolerated dose (MTD) was 6mg with dose limiting toxicity (DLT) of grade 3 rash in 1 out of 6 patients[55]. In this study, pharmacodynamic (PD) biomarker measurements in peripheral blood mononuclear cells, skin, and tumor showed treatment-related changes, occurring even at low exposures, as evidenced by decreases in p4EBP1, pS6, and pPRAS40.

The pharmacokinetic (PK) profile of MLN0128 has been evaluated in 130 patients in several ongoing studies[22]. MLN0128 is rapidly absorbed after oral administration achieving a Cmax within 2 to 4 hours post-dose. MLN0128 exhibited dose-linear PK with an elimination half-life (t1/2) of approximately 8 hours. The mean steady-state plasma Cmax ranged from 52-232 nM. No significant change in the PK parameters of MLN0128 was observed on repeat dosing; the mean accumulation index of MLN0128 ranged from 0.7-1.7-fold following multiple doses.

Combinations of mTOR and tyrosine kinase or antibody VEGF/VEGFR inhibitors have been studied in a variety of cancers[56]. A phase II trial of combination bevacizumab and temsirolimus was conducted in patients with recurrent or persistent endometrial cancer[57]. Twelve patients (24.5%) experienced a RECIST response including one complete response and 23 patients (46.9%) were progression free at six months. The increased response with the combination did come with a cost of increased toxicity including intestinal perforations (2), fistula formation (2) and a grade 4 thromboembolic event. The combination of bevacizumab with sorafenib had a remarkably high response rate (47%) in patients with EOC in a phase 1 trial[5].
Moreover, combining VEGF and mTOR inhibitors has demonstrated greater tumor growth inhibition and better survival compared to either agent alone in additional preclinical studies utilizing intra-cranial glioma models[58].

In xenograft models of breast cancer with transgenically elevated VEGF levels, the combination of INK128 and bevacizumab produced superior target inhibition and inhibition of tumor growth compared to either INK128 or bevacizumab alone[22,59]. Target (TORC1 and TORC2) inhibition

was evidenced by reduced levels of S6pSer240/244, 4EBP1pThr37/45 and AKTpSer473.

Based on these studies, dual inhibition of the VEGF/VEGFR pathway with effective inhibition of mTOR signaling represents an attractive combination for a study in advanced solid tumors including recurrent GBM, ovarian and endometrial cancers.

The combination of MLN0128 with bevacizumab may have more antitumor activity than either agent alone. This phase I study will examine the safety and tolerability of the combination and determine the MTD and recommended phase II dose (RP2D) in patients with solid tumors, including GBM.

2.5 Correlative Studies Background

Plasma and CSF MLN0128 concentrations: Stage 2 recurrent GBM patients
We will assess plasma and CSF concentrations of MLN0128 in the presence or absence of bevacizumab in patients with recurrent GBM. Evaluation of achievable drug levels particularly in presence of bevacizumab will provide critical insight into whether bevacizumab co-administration should be considered in a follow-up Phase II study. The concentration of MLN0128 in study samples will be determined by validated LC-MS/MS assay.

Archival tumor analysis: Stage 2 recurrent GBM patients
Archival paraffin-embedded tumor will be analyzed for markers associated with dysregulated cell signaling that may predict response to mTOR inhibitor therapy such as EGFR (amplification by FISH), PTEN (expression by IHC and deletion by FISH), p-AKT, p-S6, p-4EBP, p-mTOR and p-ERK in patients with recurrent GBM.

Tumor biopsies: Stage 2 endometrial and ovarian cancer patients
Endometrial and ovarian cancer patients in stage 2 will undergo serial tumor biopsies to characterize the effects that MLN0128 has upon the tumor and associated stroma. Tumor biopsies will be obtained through Interventional Radiology only among patients considered at minimal surgical risk. Biopsies will be obtained before treatment, after 2 weeks of MLN0128 monotherapy , and optionally at the time of progression on study therapy. This plan will allow the discovery of possible predictive biomarkers that can be queried in future trials with this therapeutic regimen and as well for pharmacodynamic endpoints associated with MLN0128 therapy.

Circulating cytokines in blood: Stage 2 endometrial and ovarian cancer patients
We will assess plasma for the following cytokines, VEGFA, PIGF, bFGF, , VEGFD, MMP2, MMP9, IL6, IL8, EGF and angiopoietin. Cytokine selection is based upon published reports of proteins and growth factors believed to be important in vascular formation (VEGFA,VEGFD, IL6, IL8, Angiopoietin-1, bFGF), tumor aggressiveness and invasion (IL-6, IL-8, EGF, MMP2, MMP9), resistance/sensitivity to bevacizumab mediated therapy (VEGFA, VEGFD, IL-6, Angiopoietin-1), or linked with signaling through PI3K/AKT/mTOR pathway. These angiogenic factors will be assessed as markers for response, resistance or progression.

3. PATIENT SELECTION

Participants must meet the following eligibility criteria on screening examination to be eligible to participant in the study. Screening evaluations are detailed in Section 10.

Following registration, on Cycle 1, Day 1, participants should be evaluated for eligibility to treat based upon dose modification criteria outlined in Table 6.1 and 6.2 and not inclusion and exclusion criteria outlined here in Protocol Sections 3.1 and 3.2; these sections need only be met during screening for trial for registration.

3.1 Eligibility Inclusion Criteria

3.1.1 Patients must have a histologically/cytologically confirmed diagnosis of recurrent glioblastoma or an advanced solid tumor in which bevacizumab has shown benefit in specific disease population and for which standard or curative measures do not exist or are no longer effective.

3.1.2 Measurable or evaluable disease as assessed by RECIST 1.1 for non-GBM tumors and by RANO criteria for GBM[60,61].

3.1.3 For Stage 1 (all patients) and dose expansion (stage 2) endometrial and ovarian cancer cohorts, participants are allowed following unlimited prior therapy. For Stage 2 GBM participants, no more than 2 prior relapses are allowed. For these patients, relapse is defined as progression following initial therapy (i.e. radiation +/- chemo if that was used as initial therapy) or a subsequent therapy. The intent therefore is that GBM patients enrolling onto stage 2 had no more than 3 prior therapies (initial and treatment for 2 relapses). If the patient had a surgical resection for relapsed disease and no anti-cancer therapy was instituted for up to 12 weeks, and the patient undergoes another surgical resection, this is considered to constitute 1 relapse.

NOTE: For participants who had prior therapy for a low-grade glioma, the surgical diagnosis of glioblastoma will be considered the first relapse. Therefore, these participants may have had more than 3 prior therapies.

3.1.4 Patients must have recovered from clinically significant toxicity of prior therapy to grade ≤ 1 or pre-treatment baseline. The following intervals from previous treatments are required prior to day 1 of study therapy:

- 12 weeks from the completion of radiation for recurrent GBM unless there is surgical diagnosis of recurrence or a new lesion that was not previously radiated
- 6 weeks from a nitrosourea chemotherapy
- 3 weeks from a non-nitrosourea chemotherapy
- 4 weeks from an investigational agent (not FDA approved) (or 5 half lives, whichever is shorter)
- 2 weeks from administration of a non-cytotoxic, FDA-approved agent (e.g., erlotinib, hydroxychloroquine, etc.) (or 5 half lives, whichever is shorter).

3.1.5 Age ≥18 years.

3.1.6 ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A).

3.1.7 Patients must have normal organ and marrow function as defined below:

• Absolute neutrophil count	≥ 1,500/μL
• Platelets	≥ 100,000/μL
• Hemoglobin	≥ 9.0g/dL
• Total bilirubin	<1.5 x institutional upper limit of normal with direct bilirubin within normal limits except for participants with Gilbert's disease
• AST(SGOT)/ALT (SGPT)	≤ 2.5 x institutional upper limit of normal (≤ 5 x ULN if liver metastases are present).
• Creatinine	< 1.5 x normal institutional limits **OR**
• Creatinine clearance	≥ 50mL/min/1.73m^2 for patients with creatinine level above institutional normal based either on Cockroft-Gault estimate or based on urine collection (12 or 24 hour).

3.1.8 Metabolic: fasting serum glucose (≤ 130 mg/dL) and fasting triglycerides ≤ 300 mg/dL

3.1.9 The effects of MLN0128 on the developing human fetus are unknown. For this reason and because mTOR inhibitors as well as other therapeutic agents (bevacizumab) used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, the duration of study participation and 6 months after completion of MLN 0128 or bevacizumab administration. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 6 months after completion of MLN0128 or bevacizumab administration.

3.1.10 Patients must have no concurrent malignancy except curatively treated basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix, breast, or bladder. Patients with prior malignancies must be disease-free for ≥ three years prior to registration.

3.1.11 Solid tumor patients must be off corticosteroids prior to registration. If GBM patient is receiving corticosteroids, patient must be on a stable or decreasing dose of corticosteroids for at least 5 days prior to baseline MRI or CT. If steroids are added or the steroids dose is increased between the date of the screening MRI or CT and the start of treatment, a new baseline MRI or CT is required.

3.1.12 Patients must be able to swallow whole capsules.

3.1.13 Ability to understand and the willingness to sign a written informed consent document.

3.1.14 For Stage 2 GBM participants, a block of paraffin embedded tissue or 30 unstained slides at standard 4-5 um thickness from any prior surgery demonstrating GBM pathology must be available for submission.

3.1.15 Stage 2 endometrial and ovarian cancer patients must have at least one lesion amenable to biopsy. This determination will be made by a member of the interventional radiology team or surgical associate investigator and an associate investigator. This requirement is not necessary for patients in stage 1.

3.1.16 Solid tumor patients in stage 2 must have a diagnosis of papillary serous, endometrioid or clear cell endometrial carcinoma or, high grade serous, clear cell, endometrioid or mucinous ovarian, fallopian or primary peritoneal carcinoma.

3.2 Eligibility Exclusion Criteria

3.2.1 Concurrent administration of any other investigational agents.

3.2.2 History of allergic reactions attributed to compounds of similar chemical or biologic composition to MLN0128 or bevacizumab. The MLN0128 or Bevacizumab Investigator Brochure can be referenced for more information.

3.2.3 For all Stage 2 participants, no prior treatment with mTOR, PI3 kinase or Akt inhibitors. Prior treatment with mTOR, PI3 kinase or Akt inhibitors allowed in Stage 1 only.

3.2.4 For Stage 2 GBM participants, no prior treatment with bevacizumab/VEGFR inhibitors. Prior treatment with bevacizumab/VEGFR inhibitors is allowed in Stage 1 for all participants, as well as Stage 2 endometrial and ovarian cancer participants.

3.2.5 Stage 1 solid tumor and stage 2 endometrial and ovarian cancer participants with known CNS metastatic lesions which are symptomatic and/or growing. Patients previously treated for these conditions that are asymptomatic in the absence of corticosteroid therapy are allowed to enroll. Brain metastasis must be stable for 1 month with verification by imaging (brain MRI completed at screening demonstrating no current evidence of progressive brain metastases). CNS imaging will not be mandated for asymptomatic patients with no history of CNS metastases.

3.2.6 Concurrent use of enzyme-inducing anti-epileptic drugs (EIAED). Patients may be on non-enzyme inducing anti-epileptic drugs or not be taking any anti-epileptic drugs. Patients previously treated with EIAED may be enrolled if they have been off the EIAED for 10 days or more prior to the first dose of MLN0128. (see Section 5.3, Prohibited Medication During Study).

3.2.7 Subjects taking strong CYP3A4 and CYP2C19 inhibitors and/or inducers should be considered with caution. Alternative treatments that are less likely to affect MLN0128 metabolism, if available, should be considered. If a subject requires treatment with 1 or more of the strong CYP3A4 and CYP2C19 inhibitors and/or inducers, the Principal Investigator should be consulted. Examples of the strong CYP3A4 and CYP2C19 inhibitors and inducers included in Appendix C).

3.2.8 Concurrent use of herbal supplements and other non-traditional medications. All herbal supplements and other non-traditional medications must be stopped before time of registration.

3.2.9 Concurrent use of anti-coagulants (warfarin, etc.) other than low-molecular weight heparin (LMWH). Medication must be stopped before time of registration. If patient has recently been on anti-coagulants other than LMWH, patient must have INR ≤ 2.

3.2.10 Evidence of any significant intracranial hemorrhage, as determined by the treating investigator, within 6 weeks from registration or as seen on most recent MRI prior to screening/baseline MRI.

3.2.11 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements, are ineligible.

3.2.12 History of any of the following within 6 months prior to start of MLN0128:

- Left ventricular ejection fraction (LVEF) ≤ 55 % as determined by MUGA scan or ECHO
- Heart failure ≥ NYHA grade 3
- Significant ST depression of ≥ 1.5 mm in 2 or more leads and/or T wave inversions in ≥ 2 leads
- Complete left bundle branch block
- Right bundle branch block + left anterior hemiblock (bi-fascicular block)
- Congenital long QT syndrome
- QTcF > 450 msec on screening ECG
- Requirement of inotropic support (excluding digoxin)
- History or presence of clinically significant ventricular or atrial tachyarrhythmias, or cardiac arrest
- Clinically significant resting bradycardia
- Presence of unstable atrial fibrillation (ventricular response >100 beats per minute).
- Patients with stable atrial fibrillation are allowed in the study provided they do not meet the other cardiac exclusion criteria
- History of arrhythmia requiring an implantable cardiac defibrillator
- Angina pectoris ≤ 12 months prior to starting drug
- Acute myocardial infarction ≤ 12 months prior to starting drug
- Any valve disease CTCAE grade
- Ischemic myocardial event including angina requiring therapy and artery revascularization procedures
- Placement of a pacemaker for control of rhythm
- Pulmonary embolism
- Ischemic cerebrovascular event, including TIA and artery revascularization procedures

3.2.13 Impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of oral MLN0128 (e.g., ulcerative diseases, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, small bowel resection that requires nutritional support).

3.2.14 Use of hematopoietic colony-stimulating growth factors (e.g. G-CSF, GMCSF, M-CSF) ≤ 2 weeks prior to starting study drug. Erythropoietin, darbepoetin and Erythropoietin-biosimilars are allowed for as long as they have been initiated at least 2 weeks prior to study enrollment.

3.2.15 Pregnant or nursing women. MLN0128 has potential for teratogenic or abortifacients effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with MLN0128, breastfeeding should be discontinued if the mother is treated with MLN0128.

3.2.16 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with MLN0128. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. If an HIV-positive patient has adequate CD4 counts (CD4 above the lower limit of institutional normal) and is on antiretroviral therapy with newer agents, which are not strong CYP inhibitors (see Appendix C), they will be eligible.

3.2.17 Uncontrolled high blood pressure (i.e., systolic blood pressure ≥ 160 mmHg, diastolic blood pressure ≥ 90mmHg)

3.2.18 Pulmonary hypertension

3.2.19 Uncontrolled asthma or O2 saturation < 90% by ABG (arterial blood gas) analysis or pulse oximetry on room air

3.2.20 Participants with poorly controlled diabetes mellitus (defined as HbA1c >7%); subjects with a history of transient glucose intolerance due to corticosteroid administration are allowed in this study if all other inclusion/exclusion criteria are met.

3.2.21 Urine protein should be screened by urinalysis. If protein is 2+ or higher, 24 hour urine protein should be obtained and the level should be <1000mg for patient enrollment.

3.2.22 Serious or non-healing wound, ulcer or bone fracture.

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

3.2.24 Invasive procedures defined as follows:

- Major surgical procedure, open biopsy or significant traumatic injury within 28 days prior to day 1 therapy.

- Anticipation of need for major surgical procedures during the course of the study.
- Core biopsy within 7 days prior to day 1 therapy

3.2.25 Significant vascular disease (e.g. aortic aneurysm requiring surgical repair or recent peripheral arterial thrombosis) within 6 months prior to day 1.

3.2.26 Evidence of bleeding diathesis or coagulopathy.

3.2.27 Patients with known hypersensitivity to Chinese Hamster ovary cell products or other recombinant human antibodies.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

Eligible patients will be entered on study centrally at the Dana-Farber Cancer Institute by the Data Manager. All sites should call the Data Manager, Katrina Smith at 617-632-3780 to verify dose level availabilities. Following registration, patients should begin protocol treatment within 5 days. Issues that would cause treatment delays should be discussed with the Principal Investigator, Dr. Lakshmi Nayak. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Coordinating Center should be notified of cancellations as soon as possible.

4.2 Registration Process

To register patients onto this study please refer to Appendix D, Data and Safety Monitoring Plan (DSMP) Section 3.7.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Stage 1: **Dose escalation to estimate MTD/RP2D**.
Approximately 12-18 patients with advanced solid tumors including recurrent GBM will be

enrolled using a standard 3+3 design, including 3-6 patients in each cohort. Patients will start at dose level 1, 3mg oral daily dosing of MLN0128 based on the MTD from a prior phase I study of single agent MLN0128[55]. MTD will be defined as the highest dose at which <2/6 patients will experience a DLT. The dose of bevacizumab will be 10mg/kg every 2 weeks.

Note: A pilot study evaluating perioperative MLN0128 dosing in recurrent GBM patients (NCI 9546) will be ongoing to determine the optimal dosing for brain penetration. If that study determines that once-weekly dosing of MLN0128 leads to better CNS penetration among GBM patients, then the current study will be amended accordingly to include a separate dose escalation schema of weekly MLN0128 and bevacizumab among recurrent GBM patients.

Table 5.1

Stage 1 Dose Escalation Schedule		
Dose Level	**Dose**	
	MLN0128	**Bevacizumab**
Level -1	3mg oral, daily for 5 of 7 days	10mg/kg intravenously, every 2 weeks
Level 1*	3mg oral, daily	10mg/kg intravenously, every 2 weeks
Level 1a**	4mg oral, daily for 5 of 7 days	10mg/kg intravenously, every 2 weeks
Level 2	4mg oral, daily	10mg/kg intravenously, every 2 weeks
Level 3	5mg oral, daily	10mg/kg intravenously, every 2 weeks

*Starting dose level
**Enrollment will begin at Level 1 and then proceed to Level 2, if there are DLTs at Level 2 then enrollment will proceed on Level 1a as defined in Section 6.2.

Stage 2: Expansion cohort at MTD.
After the MTD/RP2D is determined, up to 30 patients with ovarian and endometrial cancer, and 10 patients with recurrent GBM will be enrolled and treated at the MTD/RP2D to: 1) better characterize the safety profile of MLN0128; 2) estimate plasma and CSF concentrations of MLN0128 when administered with and without bevacizumab; and 3) evaluate the pharmacodynamic properties of MLN0128 in tumor tissue and plasma.

5.1.1 CTEP IND Agent: MLN0128

MLN0128 is an oral drug. MLN0128 will be administered orally once (unless enrolled in dose level -1 or 1a as described above) in the morning after a light meal at approximately the same time of day. MLN0128 may be taken later on particular clinic days, in order to accommodate for research related testing. MLN0128 capsules should be swallowed with water without chewing or sucking the capsule. Capsules should not be opened. If the subject chews or sucks the capsule by error, the subject should drink a large glass of

water (~8 oz.). Subjects should be encouraged to drink at least 18-24 ounces of liquids a day to stay well-hydrated.

In cases where a subject misses dosing at his/her dosing time, the subject may still take the dose within 12 hours of the regular dosing time with a meal (subjects should not take 2 consecutive daily doses within 12 hours of each other). If subject misses a scheduled dose more than 12 hours following regular dosing time, the dose should be noted as missed in the pill diary (missed doses should not be made up for the 5 of 7 days dosing in doses level -1 or 1a). Subjects who vomit shortly after receiving MLN0128 will not receive a replacement dose. If confirmed that the study drug has been vomited, the dose should be noted as having been missed.

On days of scheduled pharmacokinetic sampling, the MLN0128 dose must be taken in the outpatient clinic (prior to bevacizumab administration), to allow for the post-dosing PK sample.

Grapefruit and grapefruit juice should be avoided while participating in this protocol.

Compliance
The participant will be requested to maintain a medication diary of each dose of the oral medication, MLN0128. The medication diary will be returned to clinic staff at the end of each course. See Appendix E.

5.1.2 Bevacizumab

Patients will be treated with bevacizumab at 10mg/kg intravenously (IV) on days 1 and 15 (+/- 2 days of each 28-day cycle with no less than 10 days between bevacizumab administrations), except for patients enrolled in stage 2 of the trial who will not receive bevacizumab on cycle 1 day 1, and will start treatment with bevacizumab on cycle 1 day 15.

The calculated dose of bevacizumab should be placed in 100 mL of 0.9% Sodium Chloride for injection. Administration will be as a continuous IV infusion. Anaphylaxis precautions should be observed during study drug administration. It is not necessary to correct dosing based on ideal weight. Actual body weight at each clinic visit prior to bevacizumab dosing (on Days 1 and 15 of each 28 day cycle) will be used to calculate the dose of bevacizumab.

The initial dose will be delivered over 90 ± 15 minutes. If the first infusion is tolerated without infusion-associated adverse events (fever and/or chills), the second infusion may be delivered over 60 ± 10 minutes. If the 60-minute infusion is well tolerated, all subsequent infusions may be delivered over 30 ± 10 minutes. If a subject experiences an infusion–associated adverse event, he or she will be monitored according to institutional guidelines and may be pre-medicated for the next study drug infusion.

Special Precautions/Safety Issues:

Prior to each treatment, the patient should be carefully assessed with special attention to BP, proteinuria, bleeding and cardiovascular events, as well as symptoms or signs of bowel perforation and RPLS. Decisions for retreatment or dose modification/interruption should follow the dose modification guidelines in Section 6.1.

Patients who have an ongoing study agent-related SAE upon study completion or at discontinuation from the study will be contacted by the investigator or his/her designee periodically until the event is resolved or determined to be irreversible.

Infusional reactions: Routine premedication is not required for the first dose of bevacizumab. If infusional reactions occur, acetaminophen, diphenhydramine, steroids, or other medications may be given for symptom control and for premedication as needed. Anaphylactic precautions should be observed during bevacizumab administration.

Hypertension: Patients should have BP monitored prior to each infusion of bevacizumab. Hypertensive mediation should be initiated or increased for optimal BP control according to standard public heath guidelines.

Proteinuria: Proteinuria should be monitored by dipstick at least every 6 weeks.

Surgery and wound complication issues and surgery: The appropriate interval from discontinuation of bevacizumab to subsequent elective surgery required to reduce the risk of impaired wound healing has not been determined. Decision on such an interval should take into consideration the half-life of bevacizumab. It is generally recommended that bevacizumab should be discontinued at least 4-8 weeks prior to major elective surgery. In addition, bevacizumab should not be restarted until at least 4 weeks after major surgery provided that the wound has adequately healed; in cases of high risk procedures such as liver resection, thoracotomy, or neurosurgery, it is recommended that bevacizumab be resumed no earlier than 8 weeks after surgery.

5.2 Definition of Dose-Limiting Toxicity

Dose-limiting toxicity (DLT) is based on the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE Version 4.0). A DLT is defined as an adverse event (AE) or abnormal laboratory value assessed as possibly, probably or definitely related to MLN0128 and/or bevacizumab study therapy that occurs during the initial 28 days of therapy **and** meets any of the following criteria:

- Grade 3 non-hematologic toxicity:
    - The following will not be considered dose limiting unless they persist despite maximal medical treatment for more than 3 days: grade 3 nausea/vomiting, electrolyte imbalance, hypertension, and hyperglycemia;
    - The following grade 3 laboratory abnormalities will not be considered dose limiting if they return to baseline within 7 days: bilirubin, AST, ALT, cholesterol

high, hypertriglyceridemia, amylase, lipase and creatinine.

- Grade 4 non-hematologic toxicity despite maximal medical therapy;
- Grade ≥ 3 thrombocytopenia, febrile neutropenia (ANC < 1.0 X 10^9/L or 1000/mm^3 and a single temperature of > 38.3°C (101°F) or a sustained temperature of ≥ 38°C (100.4°F) for more than one hour) or Grade 4 neutropenia;
- Any treatment related AEs that lead to treatment delays or skipped doses for more than 14 days would be considered a DLT.

Investigators, together with the Overall PI, Dr. Lakshmi Nayak and CTEP, can declare a DLT if a patient is experiencing increasing toxicity at any time during treatment, and it becomes clear that it is not going to be possible to complete the treatment without exposing the patient to excessive risk.

After MTD/RP2D is determined and patients are accrued in Stage 2 of the study, if 2 or more patients in each dose-expansion cohort develop unacceptable toxicity defined as grade 4 or 5 non-hematological toxicity or ≥grade 2 intracranial hemorrhage (in recurrent GBM patients), then further accrual to that cohort will be stopped and the next dose level down will be considered the MTD for that particular cohort. At that time, Dr. Lakshmi Nayak (the overall principal investigator) will discuss with CTEP and Millennium to determine if further accrual needs to occur to obtain additional information regarding toxicity.

Whenever a patient experiences toxicity that fulfills criteria for DLT, treatment with the study regimen will be interrupted and the toxicity will be followed up as described in Section 6. For the purposes of dose escalation and determination of MTD, DLTs that occur during the initial 28 days of therapy will be necessarily considered, including those in which the event started in the initial 28 days of therapy and the confirmation of DLT occurs in a subsequent cycle.

NOTE: If a Stage 1 participant comes off active study treatment for a reason other than toxicity before receiving a full cycle (28 days) of therapy, s/he is not considered evaluable for determination of the MTD. Therefore, although a Stage 1 participant who comes off active study treatment for a reason other than toxicity before receiving a full cycle (28 days) of therapy will typically be considered otherwise evaluable for study analysis purposes, an additional participant should be accrued to that cohort to replace him/her only in determination of the MTD.

Rules for re-initiation and dose modifications of MLN0128 and bevacizumab are outlined in Section 6.

Dose escalation will proceed according to the following:

Table 5.2

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level.
≥2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

5.3 General Concomitant Medication and Supportive Care Guidelines

Prohibited Concomitant Medications During Study
Oral administration of MLN0128 in humans has a low potential for metabolic and transporter-based drug-drug interactions. Therefore, except for EIAED and herbal / non-traditional medication, the use of any concomitant medication/therapies deemed necessary for the care of the patient is allowed.

Corticosteroids
GBM patients may be on stable or decreasing dose of corticosteroids (no increase for 5 days) at the time of study entry. Solid tumor, including endometrial and ovarian, cancer patients with known CNS metastatic lesions must be stable off corticosteroid therapy.

Antiemetics
The use of any antiemetic deemed necessary for the care of the patient is allowed.

Anticonvulsants
Patients may not be on enzyme-inducing anti-epileptic drugs (EIAED); patients who require anti-epileptic drugs (AED) may be on non-enzyme inducing anti-epileptic drugs (NEIAED). If a patient on this study protocol needs to have an AED started or needs to have a second AED added then only NEIAED should be used. There must be a ≥ 10 day period from discontinuation

of an EIAED and initiation of therapy. In the event that an enzyme-inducing anti-epileptic drug must be used for a patient on study the patient will be removed from the protocol.

Herbal and Non-Traditional Medications
No data exist regarding the interaction of MLN0128 with commonly used herbal or non-traditional medications. Patients should be instructed not to use such medications while receiving MLN0128 therapy.

Strong CYP3A4/CYP2C19 Inducers/Inhibitors
If a subject requires treatment with 1 or more of the strong CYP3A4 and CYP2C19 inhibitors and/or inducers, the Principal Investigator should be consulted (see Appendix C). There are no known strong specific CYP2C9 inhibitor or inducers. Examples of moderate inhibitors of CYP2C9 are carbamazepine and rifampin. These agents show some degree of overlap with their modulation of CYP3A4 and CYP2C19 activity and should hence be considered with similar caution.

5.4 Duration of Therapy

In the absence of treatment delays due to adverse event(s), study treatment may continue for until one of the following criteria applies:

- Disease progression (Section 11),
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5.5 Duration of Follow Up

Patients will be followed after removal from study treatment up until the 30-day follow-up contact or until death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

Once participants complete the 30-day follow-up period, they will be followed annually for survival via telephone or medical record follow-up until patient either passes away or is lost to follow-up. Progression information will be collected for those participants who do not progress while on study treatment.

5.6 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.4 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator**, Dr. Lakshmi Nayak,** at 617-632-2166 within 24 hours when possible.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 Bevacizumab

No dose modifications of bevacizumab are permitted. Bevacizumab dose will be based on actual weight at each clinic visit prior to bevacizumab administration on Days 1 and 15 (+/- 2 days) of each 28 day cycle. If adverse events occur that require holding bevacizumab, the dose will remain the same once treatment resumes. Patients who require bevacizumab discontinuation are permitted to continue to receive study treatment with MLN0128 alone.

Any toxicities deemed at least possibly related to bevacizumab treatment should be managed according to Table 6.1. Bevacizumab has a terminal half-life of 2 to 3 weeks; therefore, its discontinuation results in slow elimination over several months. There is no available antidote for bevacizumab.

Subjects should be assessed clinically for toxicity prior to, during, and after each infusion. If unmanageable toxicity occurs because of bevacizumab at any time during the study, treatment with bevacizumab should be discontinued.

Infusion Reaction: Infusion of bevacizumab should be interrupted for subjects who develop dyspnea or clinically significant hypotension. Subjects who experience a NCI CTCAE v. 4.0 Grade 3 or 4 allergic reaction / hypersensitivity, adult respiratory distress syndrome, or bronchospasm (regardless of grade) will be discontinued from bevacizumab treatment.

The infusion should be slowed to 50% or less or interrupted for subjects who experience any infusion-associated symptoms not specified above. When the subject's symptoms have completely resolved, the infusion may be continued at no more than 50% of the rate prior to the reaction and increased in 50% increments every 30 minutes if well tolerated. Infusions may be restarted at the full rate during the next cycle.

Adverse events requiring delays or permanent discontinuation of bevacizumab are listed in Table 6.1

Patients who require discontinuation of study bevacizumab therapy due to toxicity will be allowed to continue to receive MLN0128 study therapy unless criteria specified in Section 5.4 are met. The maximum length of bevacizumab interruption is 28 days.

Table 6.1: Bevacizumab Dose Management Due to Adverse Events

Treatment Modification for Bevacizumab-Related Adverse Events		
Event	**CTCAE. V4 Grade**	**Action to be Taken**
Allergic reactions Or **Infusion-related reactions** Or **Anaphylaxis**	Grade 1-2	• Infusion of bevacizumab should be interrupted for subjects who develop dyspnea or clinically significant hypotension. • For infusion-associated symptoms not specified above, infusion should be slowed to 50% or less or interrupted. Upon complete resolution of the symptoms, infusion may be continued at no more than 50% of the rate prior to the reaction and increased in 50% increments every 30 minutes if well tolerated. Infusions may be restarted at the full rate during the next cycle. • Subjects who experience bronchospasm (regardless of grade) should discontinue bevacizumab.
	Grade 3-4	Discontinue bevacizumab
Thromboembolic Event (Arterial), arterial ischemia - Cardiac ischemia - Myocardial infraction - CNS ischemia (TIA, CVA) - Any peripheral or visceral arterial ischemia/thrombosis	Grade 2 (new or worsening since bevacizumab)	Discontinue bevacizumab.
	Grade 3-4	Discontinue bevacizumab
Thromboembolic Event (Venous)	[Note: Patients with primary lung cancer requiring therapeutic anticoagulation should discontinue bevacizumab]	
	Grade 3 OR asymptomatic Grade 4	▪ Hold bevacizumab treatment. If the planned duration of full-dose anticoagulation is <2 weeks, bevacizumab should be held until the full-dose anticoagulation period is over. ▪ If the planned duration of full-dose anticoagulation is >2 weeks, bevacizumab may be resumed during full-dose anticoagulation **IF** <u>all</u> of the criteria below are met: - The subject must not have pathological conditions that carry high risk of bleeding (e.g. tumor involving major vessels or other conditions) - The subject must not have had hemorrhagic events while on study - The subject must be on stable dose of heparin or have an in-range INR (usually 2-3) on a stable dose of

Treatment Modification for Bevacizumab-Related Adverse Events		
Event	**CTCAE. V4 Grade**	**Action to be Taken**
		warfarin prior to restarting bevacizumab. ▪ If thromboemboli worsen/recur upon resumption of study therapy, discontinue bevacizumab
	Grade 4 (symptomatic)	Discontinue bevacizumab
Hypertension	[Treat with anti-hypertensive medication as needed. The goal of BP control should be consistent with general medical practice]	
	Grade 1 (SBP 120-139 mmHg or DBP 80-89 mmHg)	Consider increased BP monitoring; start anti-hypertensive medication if appropriate
	Grade 2 asymptomatic (SBP 140-159 mmHg or DBP 90-99 mmHg)	Start or adjust anti-hypertensive therapy and continue bevacizumab
	• Grade 2 symptomatic (SBP 140-159 mmHg or DBP 90-99 mm Hg) • Grade 3 (SBP ≥160 mmHg or DBP ≥100 mmHg)	• Start or adjust anti-hypertensive medication • Hold bevacizumab until symptoms resolve **AND** BP < 160/90mmHg • For hypertension that is refractory requiring delay of bevacizumab for > 4 weeks, discontinue bevacizumab
	Grade 4 (Hypertensive crisis or malignant hypertension)	Discontinue bevacizumab
Heart Failure OR **Left Ventricular (LV) dysfunction**	• Heart failure ≥Grade 2 • LV dysfunction ≥Grade 3	Discontinue bevacizumab
Proteinuria Proteinuria will be monitored by urine analysis dipstick. If Dipstick ≥2+ proteinuria, 24-hour urine protein should	Dipstick ≥ 2+	Hold bevacizumab and obtain 24 hour urine protein
	If 24-h urine protein <2g	Continue bevacizumab
	If 24-h urine protein ≥2 g	• Hold bevacizumab until 24-hour urine protein <2.0 g • Discontinue bevacizumab if urine protein does not recover to < 2.0 g after 28 days of bevacizumab

Treatment Modification for Bevacizumab-Related Adverse Events		
Event	**CTCAE. V4 Grade**	**Action to be Taken**
be obtained		interruption
	Nephrotic syndrome	Discontinue bevacizumab.
Hemorrhage (intracranial or pulmonary)	Grade 2-4	• Discontinue bevacizumab
	Grade 1	• Patients receiving full-dose anticoagulation should discontinue bevacizumab. • For patients not on full-dose anticoagulation, hold bevacizumab until ALL of the following criteria are met: - the bleeding has resolved and hemoglobin is stable - there is no bleeding diathesis that would increase the risk of therapy • there is no anatomic or pathologic condition that could increase the risk of hemorrhage recurrence
Hemorrhage (not CNS or pulmonary)	Grade 3	• Patients receiving full-dose anticoagulation should discontinue bevacizumab. • For patients not on full-dose anticoagulation, hold bevacizumab until ALL of the following criteria are met: - the bleeding has resolved and hemoglobin is stable - there is no bleeding diathesis that would increase the risk of therapy - there is no anatomic or pathologic condition that could increase the risk of hemorrhage recurrence. • Patients who experience recurrence of grade 3 hemorrhage should discontinue study therapy.
	Grade 4	Discontinue bevacizumab
RPLS (Reversible Posterior Leukoencephalopathy syndrome OR **PRES** (Posterior Reversible Encephalopathy Syndrome)	Any Grade	Discontinue bevacizumab upon diagnosis of RPLS/PRES.
Wound dehiscence OR **Wound complications**	Grade 2	Hold bevacizumab until healing
	Grade 3-4	Discontinue bevacizumab
Perforation (GI, or any other organ)	Any Grade	Discontinue bevacizumab

Treatment Modification for Bevacizumab-Related Adverse Events		
Event	**CTCAE. V4 Grade**	**Action to be Taken**
Fistula (GI, pulmonary or any other organ)	Any Grade	Discontinue bevacizumab
Obstruction of GI tract	Grade 2 requiring medical intervention	Hold bevacizumab until complete resolution
	Grade 3-4	• Hold bevacizumab until complete resolution • If surgery is required, patient may restart bevacizumab after 28 days and full recovery from surgery, and at investigator's discretion
Other Unspecified bevacizumab-related AEs (except controlled nausea/vomiting).	Grade 3	• Hold bevacizumab until symptoms resolve to≤Grade 1
	Grade 4	• Discontinue bevacizumab • **Upon consultation with the study chair,** resumption of bevacizumab may be considered if a patient is benefiting from therapy, and the Grade 4 toxicity is transient, has recovered to ≤Grade 1 and unlikely to recur with retreatment.

Note 1: There will be no dose reduction for bevacizumab. Treatment should be interrupted or discontinued for certain adverse events, as described below

Note 2: If bevacizumab is interrupted for ANY reasons for >4 weeks (unless otherwise specified), the patient should discontinue bevacizumab therapy on protocol.

6.2 MLN0128

For patients who do not tolerate the protocol-specified dosing schedule of MLN0128, dose adjustments are permitted in order to allow the patient to continue study treatment. Patients who require MLN0128 discontinuation due to toxicity, will be permanently discontinued from study treatment.

All dose modifications should be based on the worst preceding toxicity (CTCAE version 4.0). Dose modification of MLN0128 and/or withholding of bevacizumab will be made when toxicity is considered to be at least possibly related to the study drug. Once a dose has been reduced it will not be increased at a later time even if there is no toxicity. Adverse events (AEs) should be treated with the appropriate maximum supportive care.

Before the start of each cycle or for any AE requiring study therapy interruption or modification, drug-related toxicities must resolve to either baseline or grade 1. If a patient requires a dose delay of > 28 days from the intended day of the next scheduled dose, , then the patient should be discontinued from the study treatment. In exceptional situations, if the patient is clearly

benefiting from study drug treatment (i.e. stable disease, partial or complete response), and in the opinion of the investigator no safety concerns are present, continuation of study treatment must be discussed with the overall PI and the CTEP. Patients who require dose modification below dose level -1 (3mg/day for 5 days out of 7) will be discontinued from study therapy.

The MLN0128 dose should be reduced by one dose level* for subsequent therapy if the investigator determines that it is in the best interest of the patient or if the patient experienced at least one of the following events:

a. DLT (as defined in section 5.2)
b. DLT equivalent toxicity (cycle 2 or beyond)
c. Greater than 14 day delay to meet re-treatment criteria

***Note: Patients on dose level 2 will drop to dose level 1a if they meet any of the above criteria.**

Additional toxicity-specific dose interruption and modification guidelines are provided below in table 6.2:

Table 6.2 MLN0128 Dose Management Due to Adverse Events

Treatment Modification for MLN0128-Related Adverse Events		
Event	**CTCAE. V4 Grade**	**Action to be Taken**
CARDIAC		
Cardiac general – Left ventricular systolic dysfunction (ejection fraction decrease)	Grade 2 asymptomatic, resting ejection fraction 40-50%; or 10-19% drop from baseline	Maintain dose level of MLN0128 Repeat LVEF within 4 weeks or as clinically appropriate
	Grade 3: symptomatic responsive to intervention, ejection fraction 39-20% or > 20% drop from baseline	Omit MLN0128 dose until resolved* to normal values, • If resolved in ≤ 7 days, maintain at same dose level of MLN0128 • LVEF measurement to be repeated, if resolved * to normal values within>7 days, resume at ↓1 dose level of MLN0128. *the event is considered resolved when the patient is asymptomatic, has a resting ejection fraction ≥ 40% and ≤ 20% from baseline
	Grade 4: refractory or poorly controlled, ejection fraction < 20%	Permanently discontinue patient from the study treatment
QTc prolongation	≥ Grade 3 or > 60ms change from baseline on at least	**First Occurrence:** • Omit MLN0128 dose

Treatment Modification for MLN0128-Related Adverse Events		
Event	**CTCAE. V4 Grade**	**Action to be Taken**
	two separate ECGs	• Perform an analysis of serum potassium and mangesium, and if below lower limit of normal, correct with supplements to within normal limits. Concomitant medication usage must be reviewed. • Perform a repeat ECG within one hour of the first QTcF of 500 ms • If QTcF remains > 500 ms, repeat ECG as clinically indicated, but at least once a day until the QTcF returns to < 480 ms. Seek cardiologist input • Once QTcF prolongation has resolved, MLN0128 may be restarted at a one lower dose level **Second Occurrence:** Permanently discontinue patient from the study treatment
Other Cardiac Events	Grade 1-2	Maintain dose level of MLN0128
	Grade 3	Omit dose of MLN0128 until resolved to ≤ Grade 1 within 7 days, then resume at same dose level of MLN0128. If resolved in >7 days, resume at ↓1 dose level of MLN0128.
	Grade 4	Permanently discontinue patient from the study treatment
ENDOCRINE		
Fasting Plasma Glucose (FPG) (2 hrs fasting)	Grade 1	Maintain dose level of MLN0128, check FPG every week • Initiate or intensify with appropriate anti-diabetic treatment as per investigator's discretion • Instruct patient to follow dietary guidelines according to local and/or institutional standards for management of diabetes mellitus (such as those provided by the American Diabetes Association) during the study • Consider use of oral anti-hyperglycemic therapy such as metformin Check FGP weekly for 8 weeks, then continue checking at least every 2 weeks

Treatment Modification for MLN0128-Related Adverse Events		
Event	**CTCAE. V4 Grade**	**Action to be Taken**
	Grade 2	**First Occurrence:** If signs or symptoms of hyperglycemia (for example, mental status changes, excessive thirst, polyuria), omit dose of MLN0128 immediately and manage as for Grade 3 hyperglycemia (below) If asymptomatic, maintain MLN0128 dose and re-check FPG within 24 hours. If grade worsens or improves then follow specific grade recommendations. If FPG remains at Grade 2: • Maintain dose level of MLN0128 • Initiate or intensify with appropriate anti-diabetic treatment • Instruct patient to follow dietary guidelines according to local and/or institutional standards for management of diabetes mellitus (such as those provided by the American Diabetes Association) during the study • Consider use of oral anti-hyperglycemic therapy such as metformin If FPG does not resolve to ≤ Grade 1 within 7 days after initiation/intensifying anti-diabetic treatment: • Omit dose of MLN0128 if not already done • Monitor FPG at least weekly until FPG resolves to ≤ Grade 1 • Then re-start MLN0128 and ↓1 dose level • Continue with anti-diabetic treatment • Check FPG weekly for 8 weeks, then continue checking every 2 weeks **Second Occurrence:** Maintain dose level of MLN0128, re-check FPG within 24 hours. If grade worsens or improves then follow specific grade recommendations. If FPG remains at Grade 2: • Omit dose of MLN0128 • Initiate or intensify with appropriate anti-diabetic treatment

Treatment Modification for MLN0128-Related Adverse Events		
Event	**CTCAE. V4 Grade**	**Action to be Taken**
		• Monitor FPG at least twice weekly until FPG resolves to ≤ Grade 1 • Then re-start MLN0128 and ↓1 dose level • Continue with anti-diabetic treatment Check FPG at least weekly for 8 weeks, then continue checking at least every 2 weeks
	Grade 3	Immediately omit MLN0128, initiate or intensify medication with appropriate anti-diabetic treatment, re-check FPG within 24 hours. If grade worsens or improves then follow specific grade recommendations. If FPG remains at Grade 3: • Administer intravenous hydration and intervention for electrolyte/ketoacidosis/hyperosmolar disturbances as clinically appropriate • Continue to omit dose of MLN0128 • Monitor FPG at least twice weekly until FPG resolves to ≤ Grade 1 • If FPG resolves to ≤ Grade 1 in 7 days or less, then re-start MLN0128 at the same dose level If FPG remains greater than Grade 1 severity for more than 7 days, then discontinue patient from study treatment: • Initiate or continue anti-diabetic treatment as appropriate • Instruct patient to follow dietary guidelines according to local and/or institutional standards for management of diabetes mellitus (such as those provided by the American Diabetes Association) during the study • Consider use of oral anti-hyperglycemic therapy such as metformin • Check FPG at least weekly for 8 weeks, then continue checking at least every 2 weeks For non-fasting plasma glucose >250-500mg/dL (> 13.9 – 27.8 mmol/L) accompanied by signs/symptoms of hyperglycemia (for example, mental status changes, excessive thirst, polyuria), or presence of blood or urine ketones, omit MLN0128 and follow guidance for management of Grade 3 fasting plasma glucose (FPG).

Treatment Modification for MLN0128-Related Adverse Events		
Event	**CTCAE. V4 Grade**	**Action to be Taken**
	Grade 4	Immediately omit dose of MLN0128, initiate or intensify medication with appropriate anti-diabetic treatment, re-check within 24 hours, if confirmed Grade 4: • Administer intravenous hydration and intervention for electrolyte/ketoacidosis/hyperosmolar disturbances as clinically appropriate • Discontinue patient from the study. Instruct patient to follow dietary guidelines according to local and/or institutional standards for management of diabetes mellitus (such as those provided by the American Diabetes Association) during the study • Consider use of oral anti-hyperglycemic therapy such as metformin • Check FPG weekly for 8 weeks, then continue checking at least every 2 weeks if clinically indicated For non-fasting plasma glucose >500 mg/dL (> 27.8 mmol/L) accompanied by signs/symptoms of hyperglycemia (for example, mental status changes, excessive thirst, polyuria), or presence of blood or urine ketones, discontinue MLN0128 and following guidance for management of Grade 4 fasting plasma glucose (FPG).
GASTROINTESTINAL		
Mucositis oral	Grade 1	Maintain MLN0128 dose level
	Grade 2	Maintain MLN0128 dose level if tolerable, if toxicity becomes intolerable omit dose of MLN0128 until resolved to Grade $\leq$ 1, then restart at the same dose level
	Grade 3	Omit dose of MLN0128 until resolved to Grade $\leq$ 1, then restart at $\downarrow$1 dose level of MLN0128
	Grade 4	Permanently discontinue patient from the study treatment
GENERAL DISORDERS		
Fatigue (asthenia)	Grade 1-2	Maintain MLN0128 dose level

Treatment Modification for MLN0128-Related Adverse Events		
Event	**CTCAE. V4 Grade**	**Action to be Taken**
	Grade 3	Omit dose of MLN0128 until resolved to ≤ Grade 1, then: • If resolved in ≤ 7 days, maintain dose level of MLN0128 • If resolved in > 7 days, ↓ 1 dose level of MLN0128
	Grade 4	Omit dose of MLN0128 and discontinue patient from the study treatment
HEMATOLOGIC		
Neutropenia (ANC)	Grade 1-2	Maintain dose level of MLN0128
	Grade 3	Omit dose of MLN0128 until resolved to ≤ Grade 1 or baseline, then: • If resolved in ≤ 7 days, maintain dose level of MLN0128 • If resolved in > 7 days, resume treatment at ↓1 dose level of MLN0128
	Grade 4	Permanently discontinue patient from the study treatment
Febrile Neutropenia	Grade 3	Omit dose of MLN0128 until resolved to Grade 1 or baseline, then: • If resolved in ≤ 7 days, resume treatment at ↓1 dose level of MLN0128 • If resolved in > 7 days, discontinue MLN0128
Thrombocytopenia	Grade 1-2	Maintain dose level of MLN0128
	Grade 3	Omit dose of MLN0128 until resolved to ≤ Grade 1 or baseline then: • If resolved in ≤ 7 days, resume treatment at ↓ 1 dose level of MLN0128 • If resolved in > 7 days, discontinue MLN0128
	Grade 4	Permanently discontinue patient from the study treatment
HEPATIC		
Bilirubin	Grade 1	Maintain dose level of MLN0128
	Grade 2 (with ALT or AST grade 1 or 2)	Omit dose of MLN0128 until resolved to ≤ Grade 1 or baseline, then resume at the same dose level of MLN0128

Treatment Modification for MLN0128-Related Adverse Events		
Event	**CTCAE. V4 Grade**	**Action to be Taken**
	Grade 3 (with ALT or AST grade 1 or 2)	Omit dose of MLN0128 until resolved to ≤ Grade 1 or baseline, then: • If resolved in ≤ 7 days, maintain same dose level of MLN0128 • If resolved in > 7 days, resume treatment at ↓ 1 dose level of MLN0128
	Grade 4	Permanently discontinue patient from the study treatment
AST or ALT	Grade 1 or Grade 2 (without total bilirubin elevation > grade 1)	Maintain dose level of MLN0128
	Grade 3 (without total bilirubin elevation > grade 1)	Omit dose of MLN0128 until resolved to ≤ Grade 1 or baseline, then: • If resolved in ≤ 7 days, maintain dose level of MLN0128 • If resolved in > 7 days, resume treatment at ↓1 dose level of MLN0128
	Grade 4	Omit dose of MLN0128 until resolved to ≤ Grade 1 or baseline, then resume at ↓1 dose level
INVESTIGATIONS		
Cholesterol high and/or hypertriglyceridemia	Grade 1	Maintain MLN0128 dose level
	Grade 2	Maintain dose if tolerable, if toxicity becomes intolerable, omit dose MLN0128 until resolved to ≤ 1, then restart MLN0128 as the same dose level
	Grade 3	Omit dose of MLN0128 until resolved to Grade ≤ 1, then restart at ↓1 dose level of MLN0128
	Grade 4	Permanently discontinue patient from the study treatment
PANCREATIC		
Amylase or Lipase	Grade 1-2	Maintain dose level of MLN0128
	Grade 3	Omit dose of MLN0128 until resolved to ≤ Grade 1 or baseline, then: • If resolved in ≤ 7 days, maintain dose level of MLN0128 • If resolved in > 7 days, resume treatment at ↓1 dose level of MLN0128

Treatment Modification for MLN0128-Related Adverse Events		
Event	**CTCAE. V4 Grade**	**Action to be Taken**
	Grade 4	Permanently discontinue patient from the study treatment
RENAL		
Serum Creatinine	Grade 1	Maintain dose level of MLN0128
	Grade 2	Omit dose of MLN0128 until resolved to ≤ Grade 1 or baseline, then resume treatment at the same dose level. Patients will be instructed to increase their fluid intake until resolution to ≤ Grade 1 or baseline.
	Grade 3	Omit dose of MLN0128 until resolved to ≤ Grade 1 or baseline then: • If resolved in ≤ 7 days, maintain dose level of MLN0128 • If resolved in > 7 days, resume treatment at ↓ 1 dose level of MLN0128
RESPIRATORY		
Non-infectious Pneumonitis	Grade 1	Maintain dose level of MLN0128
	Grade 2	Omit dose of MLN0128 until resolved to Grade ≤ 1, then: • Restart at ↓1 dose level of MLN0128 • Discontinue MLN0128 if failure to recover within 4 weeks
	Grade 3	Omit dose of MLN0128 until resolved to Grade ≤ 1, then: • Consider re-initiating MLN0128 at ↓1 dose level of MLN0128 • If toxicity recurs at Grade 3, discontinue MLN0128
	Grade 4	Permanently discontinue patient from the study treatment
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
Rash (maculo-papular)	Grade 1	Maintain dose level of MLN0128. Consider to initiate appropriate skin toxicity therapy (such as antihistamines, topical corticosteroids)
	Grade 2	Maintain dose level of MLN0128. Initiate/intensify appropriate skin toxicity therapy (such as antihistamines, topical corticosteroids)
	Grade 3	Omit dose of MLN0128 until resolved to Grade ≤ 1, then: • If resolved in ≤ 7 days, maintain at same dose of MLN 0218 • If resolved in > 7 days, resume at ↓1 dose level

Treatment Modification for MLN0128-Related Adverse Events		
Event	**CTCAE. V4 Grade**	**Action to be Taken**
		of MLN0128
	Grade 4	Permanently discontinue patient from the study treatment
OTHER ADVERSE EVENTS		
Other AEs	Grade 1-2	Maintain MLN0128
	Grade 3	Omit dose of MLN0128 until resolved to ≤Grade 1, then: • If resolved in ≤ 7 days, maintain at same dose of MLN 0218 • If resolved in > 7 days, resume at ↓1 dose level of MLN0128
	Grade 4	Permanently discontinue patient from the study treatment **Note:** Omit dose for ≥ Grade 3 vomiting or Grade 3 nausea only if the vomiting or nausea cannot be controlled with optimal antiemetic therapy

6.3 Management of Hematologic Toxicities and Use of Hematologic Growth Factors

Because of the increased risk of bleeding associated with bevacizumab, patients are encouraged to receive a transfusion when their platelets reach ≤ 30 x 10^9/L.

No growth factors (G-CSF or GM-CSF) are to be used prophylactically in this protocol. Clinicians caring for patients on this protocol are permitted to use these growth factors to provide optimal care for patients with severe neutropenia in accordance with the ASCO guidelines[62]. If these growth factors are used in the acute setting of neutropenia and infection (documented or suspected), they will not be utilized prophylactically in subsequent cycles and they will not subsequently be used in lieu of dose reduction of (study agent).

Erythrocyte growth factors (e.g. erythropoietin) are not to be administered prophylactically but may be prescribed per institutional guidelines for anemia if this is
thought to be appropriate. Transfusions are also permitted.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

7.1 General Information

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. Adverse events experienced by participants will be collected, recorded and reported from the point the participant starts taking study drug up until 30 days after the last dose of study medication. The period for collection and recording of adverse events is extended for participants with ongoing reportable adverse events at least possibly related to study agent; for such participants, adverse events will be followed until the reportable event has resolved, stabilized, or determined to be irreversible by the participating investigator.

Participants should be instructed to report any serious **post-study event(s)** that might reasonably be related to participation in this study. The investigator should notify the local IRB, the Overall PI Dr. Lakshmi Nayak and any other applicable regulatory agency of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

Each adverse event will be assessed to determine if it meets the criteria for reporting. Adverse event reporting is to occur according the site's specific IRB guidelines, and as outlined in this Section for CTEP-AERS.

7.1.1 Definitions

7.1.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting study drug, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

7.1.1.2 Serious Adverse Event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death.
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form).
- Requires or prolongs inpatient hospitalization for ≥ 24 hours. Hospitalization admissions and/or surgical operations scheduled to occur during the stud period, but planned prior to signing the informed consent for the study are not considered SAEs if the illness or disease existed before the participant was

enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect.
- Is an important medical event (IME) when, based upon appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

7.1.1.3 **Expectedness**

Expected adverse events are those that have been previously identified as resulting from administration of the agent(s). For the purposes of this study, refer to the CAEPR Lists in the following sections.

- The Specific Protocol Exceptions to Expedited Reporting (SPEER) within each CAEPR list will determine whether the event requires expedited reporting via CTEP-AERS.

7.1.2 **Adverse Event Characteristics**

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

For expedited reporting purposes only:
AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.

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

7.2 **Comprehensive Adverse Events and Potential Risks (CAEPR) Lists**

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset of AEs, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column

and is identified with ***bold*** and ***italicized*** text. The SPEER is a list of events that are protocol-specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below).

Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/default.htm#adverse_events_adeers for further clarification.

NOTE: The highest grade currently reported is noted in parentheses next to the AE in the SPEER. Report **ONLY** AEs higher than this grade expeditiously via CTEP-AERS . If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

7.2.3 CAEPR for MLN0128

Adverse events that have a reasonably causal relationship to treatment with MLN0128 are indicated in the table below. Frequency is provided based on 252 patients.

Version 2.1, June 1, 2015[1]

Adverse Events with Possible Relationship to MLN0128 (INK128) (CTCAE 4.0 Term) [n= 252]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 2)***
CARDIAC DISORDERS			
		Cardiac arrest	
		Ventricular fibrillation	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
Constipation			***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 2)***
	Dry mouth		***Dry mouth (Gr 2)***
Mucositis oral			***Mucositis oral (Gr 2)***
Nausea			***Nausea (Gr 2)***
Vomiting			***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
	Fever		***Fever (Gr 2)***
	General disorders and administration site conditions - Other (mucosal inflammation)		***General disorders and administration site conditions - Other (mucosal inflammation) (Gr 2)***
INFECTIONS AND INFESTATIONS			
	Lung infection		
	Urinary tract infection		***Urinary tract infection (Gr 2)***
INVESTIGATIONS			
	Creatinine increased		***Creatinine increased (Gr 2)***
		Electrocardiogram QT corrected interval prolonged	

Adverse Events with Possible Relationship to MLN0128 (INK128) (CTCAE 4.0 Term) [n= 252]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Lymphocyte count decreased		
	Neutrophil count decreased		
	Platelet count decreased		***Platelet count decreased (Gr 2)***
	Weight loss		***Weight loss (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
Anorexia			***Anorexia (Gr 2)***
	Dehydration		***Dehydration (Gr 2)***
Hyperglycemia			***Hyperglycemia (Gr 2)***
	Hypocalcemia		
	Hypokalemia		***Hypokalemia (Gr 2)***
	Hypomagnesemia		***Hypomagnesemia (Gr 2)***
	Hypophosphatemia		***Hypophosphatemia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Back pain		***Back pain (Gr 2)***
	Pain in extremity		
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Dysgeusia		***Dysgeusia (Gr 2)***
	Headache		***Headache (Gr 2)***
RENAL AND URINARY DISORDERS			
	Acute kidney injury		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
Pruritus			***Pruritus (Gr 2)***
Rash maculo-papular			***Rash maculo-papular (Gr 2)***

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

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

Also reported on MLN0128 (INK128) trials but with the relationship to MLN0128 (INK128) still undetermined:

EYE DISORDERS - Photophobia
GASTROINTESTINAL DISORDERS - Dyspepsia; Esophagitis; Gastroesophageal reflux disease
INFECTIONS AND INFESTATIONS - Sepsis; Skin infection
INVESTIGATIONS - Alanine aminotransferase increased; Aspartate aminotransferase increased; Cholesterol high; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Hypoalbuminemia; Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Musculoskeletal and connective tissue disorder - Other (muscle spasms); Myalgia

NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Treatment related secondary malignancy
PSYCHIATRIC DISORDERS - Anxiety; Depression; Insomnia; Personality change
RENAL AND URINARY DISORDERS - Hematuria
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Epistaxis; Hypoxia; Pleural effusion
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Rash acneiform; Urticaria
VASCULAR DISORDERS - Flushing; Hypotension; Thromboembolic event

Note: MLN0128 (INK128) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2.4 CAEPR for Bevacizumab

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Bevacizumab (rhuMAb VEGF, NSC 704865)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 3540 patients.* Below is the CAEPR for bevacizumab (rhuMAb VEGF).

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

Version 2.4, May 23, 2016[1]

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

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

Neutrophil count decreased			***Neutrophil count decreased (Gr 3)***
	Platelet count decreased		***Platelet count decreased (Gr 4)***
	Weight loss		***Weight loss (Gr 3)***
	White blood cell decreased		***White blood cell decreased (Gr 3)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 3)***
	Dehydration		***Dehydration (Gr 3)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr 3)***
	Musculoskeletal and connective tissue disorder - Other (bone metaphyseal dysplasia)[11]		
	Myalgia		***Myalgia (Gr 3)***
	Osteonecrosis of jaw[12]		
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Headache		***Headache (Gr 3)***
		Intracranial hemorrhage	
		Ischemia cerebrovascular[2]	
	Peripheral sensory neuropathy[13]		
		Reversible posterior leukoencephalopathy syndrome	
	Syncope		
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
	Hematuria		***Hematuria (Gr 3)***
	Proteinuria		***Proteinuria (Gr 2)***
		Renal and urinary disorders - Other (Nephrotic Syndrome)	
		Urinary fistula	
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			
Reproductive system and breast disorders - Other (ovarian failure)[14]			
		Vaginal fistula	
	Vaginal hemorrhage		***Vaginal hemorrhage (Gr 3)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Allergic rhinitis		***Allergic rhinitis (Gr 3)***
		Bronchopleural fistula	
		Bronchopulmonary hemorrhage	
	Cough		***Cough (Gr 3)***
	Dyspnea		***Dyspnea (Gr 2)***
	Epistaxis		***Epistaxis (Gr 3)***
	Hoarseness		***Hoarseness (Gr 3)***
		Respiratory, thoracic and mediastinal disorders - Other (nasal-septal perforation)	
		Respiratory, thoracic and mediastinal disorders - Other (tracheo-esophageal fistula)	

SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Pruritus		***Pruritus (Gr 2)***
	Rash maculo-papular		***Rash maculo-papular (Gr 2)***
	Urticaria		***Urticaria (Gr 2)***
VASCULAR DISORDERS			
Hypertension			***Hypertension (Gr 3)***
	Thromboembolic event		***Thromboembolic event (Gr 3)***
		Vascular disorders - Other (arterial thromboembolic event)[2,15]	

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (idiopathic thrombocytopenia purpura); Bone marrow hypocellular; Disseminated intravascular coagulation; Hemolysis
CARDIAC DISORDERS - Atrioventricular block complete; Atrioventricular block first degree; Cardiac arrest; Myocarditis; Pericardial effusion; Restrictive cardiomyopathy; Right ventricular dysfunction
EAR AND LABYRINTH DISORDERS - Ear and labyrinth disorders - Other (tympanic membrane perforation); Hearing impaired; Tinnitus; Vertigo
ENDOCRINE DISORDERS - Hyperthyroidism; Hypothyroidism
EYE DISORDERS - Blurred vision; Cataract; Dry eye; Extraocular muscle paresis; Eye disorders - Other (blindness); Eye disorders - Other (conjunctival hemorrhage); Eye disorders - Other (corneal epithelial defect); Eye disorders - Other (floaters); Eye disorders - Other (ischemic CRVO); Eye disorders - Other (macular pucker); Eye disorders - Other (transient increased IOP > or =30 mm Hg); Eye disorders - Other (vitreous hemorrhage); Eye pain; Keratitis; Optic nerve disorder; Photophobia; Retinal detachment; Retinal tear; Retinopathy; Watering eyes
GASTROINTESTINAL DISORDERS - Ascites; Chelitis; Colonic stenosis; Dry mouth; Dysphagia; Enterocolitis; Esophageal pain; Esophageal stenosis; Flatulence; Gastrointestinal disorders - Other (peritonitis); Oral pain; Pancreatitis; Proctitis; Rectal mucositis; Rectal stenosis;Typhlitis
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Death NOS; Edema face; Edema limbs; Edema trunk; Facial pain; Fever; Flu like symptoms; Gait disturbance; Injection site reaction; Localized edema; Multi-organ failure; Sudden death NOS
HEPATOBILIARY DISORDERS - Cholecystitis; Gallbladder necrosis; Gallbladder obstruction; Hepatic failure; Hepatic necrosis
INFECTIONS AND INFESTATIONS - Infections and infestations - Other (aseptic meningitis)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Arterial injury; Bruising; Burn; Dermatitis radiation; Fracture
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Blood antidiuretic hormone abnormal; CD4 lymphocytes decreased; CPK increased; Carbon monoxide diffusing capacity decreased; Electrocardiogram QT corrected interval prolonged; Forced expiratory volume decreased; GGT increased; INR increased; Lipase increased; Lymphocyte count decreased; Serum amylase increased; Weight gain
METABOLISM AND NUTRITION DISORDERS - Acidosis; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hypokalemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Back pain; Bone pain; Chest wall pain; Fibrosis deep connective tissue; Generalized muscle weakness; Head soft tissue necrosis; Joint effusion; Muscle weakness lower limb; Muscle weakness upper limb; Musculoskeletal and connective tissue disorder - Other (aseptic necrotic bone); Musculoskeletal and connective tissue disorder - Other (myasthenia gravis); Musculoskeletal and connective tissue disorder - Other (polymyalgia rheumatica); Neck pain; Pain in extremity; Pelvic soft tissue necrosis; Soft tissue necrosis lower limb
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain
NERVOUS SYSTEM DISORDERS - Arachnoiditis; Ataxia; Central nervous system necrosis; Cerebrospinal fluid leakage; Cognitive disturbance; Depressed level of consciousness; Dysesthesia;

Dysgeusia; Dysphasia; Encephalopathy; Extrapyramidal disorder; Facial nerve disorder; Hydrocephalus; Leukoencephalopathy; Memory impairment; Nervous system disorders - Other (increased intracranial pressure); Paresthesia; Peripheral motor neuropathy; Pyramidal tract syndrome; Seizure; Somnolence; Tremor; Vasovagal reaction
PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Depression; Insomnia; Libido decreased; Psychosis
RENAL AND URINARY DISORDERS - Bladder spasm; Chronic kidney disease; Cystitis noninfective; Renal and urinary disorders - Other (dysuria); Renal and urinary disorders - Other (ureterolithiasis); Renal hemorrhage; Urinary frequency; Urinary incontinence; Urinary retention; Urinary tract obstruction; Urinary tract pain
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Breast pain; Erectile dysfunction; Irregular menstruation; Pelvic pain; Vaginal discharge
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Atelectasis; Hypoxia; Nasal congestion; Pulmonary fibrosis; Pulmonary hypertension; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (dry nares); Respiratory, thoracic and mediastinal disorders - Other (pulmonary infarction)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Nail loss; Pain of skin; Palmar-plantar erythrodysesthesia syndrome; Photosensitivity; Purpura; Rash acneiform; Skin and subcutaneous tissue disorders - Other (diabetic foot ulcer); Skin and subcutaneous tissue disorders - Other (skin breakdown/ decubitus ulcer); Skin hyperpigmentation; Skin induration; Skin ulceration; Stevens-Johnson syndrome
VASCULAR DISORDERS - Flushing; Hot flashes; Hypotension; Lymphocele; Phlebitis; Vasculitis

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

7.3 Expedited Adverse Event Reporting to CTEP

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov). These requirements are briefly outlined in the tables below (Section 7.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other e-mail recipients.

7.3.3 <u>Expedited Reporting Guidelines</u>

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or

subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.3.4 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting via CTEP-AERS . However, they still must be reported through the routine reporting mechanism (Section 7.4):

CTCAE SOC	Adverse Event	Grade	Hospitalization/ Prolongation of Hospitalization	Attribution	Comments
10025256	Lymphopenia	1-4	Hospitalization/ Prolongation of Hospitalization	Any	

7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through CTEP-AERS must <u>also</u> be reported in routine study data submissions.**

7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6 Expedited Adverse Event Reporting to Local IRB

Sites within DF/HCC and DF/PCC will submit reportable adverse events directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

External participating sites will report SAEs to their respective IRB according to the local IRB's policies and procedures in reporting adverse events.

7.7 Routine Adverse Event Reporting

All Adverse Events must be reported in routine study data submissions to the Overall PI on the toxicity case report forms. AEs reported through expedited processes (e.g., reported to the IRB, CTEP-AERS , etc.) must also be reported in routine study data submissions.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 7.2.

8.1 Study Agent: MLN0128

Other Names: INK128

Classification: mTOR inhibitor, TORC1/2

CAS Registry Number: 1224844

Molecular Formula: $C_{15}H_{15}N_7O$

M.W.: 309.3

Approximate Solubility: MLN0128 exhibits a pH-dependant aqueous solubility: at physiological pH the solubility is approximately 0.1 mg/mL and at or below pH 3 the solubility is greater than 15 mg/mL.

Mode of Action: MLN0128 is a non-rapamycin analog mTOR (mammalian target of rapamycin) kinase inhibitor. The mTOR kinase regulates cell growth, translational control, angiogenesis, and cell survival by integrating nutrient and hormonal signals. The mTOR complex (TORC) is an intracellular point of convergence for a number of cellular signaling pathways. ML0128 is a potent and selective adenosine tri-phosphate (ATP)-competitive inhibitor of mTOR complex 1 and 2 (TORC1/2).

Description: MLN0128 drug substance is a white to off-white, crystalline powder.

How Supplied: MLN0128 will be supplied in tamper-resistant bottles as capsules containing 1 of 3 dose strengths.

MLN0128 capsules, 1mg – white opaque color
MLN0128 capsules, 3mg – orange opaque color
MLN0128 capsules, 5mg – grey opaque color

Each 1-, 3-, and 5-mg capsule for oral administration contain 1, 3, and 5 mg of MLN0128, respectively in addition to the following inactive ingredients: microcrystalline cellulose (solid filler/diluents), magnesium stearate (lubricant), and hard gelatin capsule.

Bottles of MLN0128 will contain 30 capsules each.

Storage: Bottles are to be stored at room temperature (15C to 30C [59F to 85F]).

Route of Administration: Oral

Potential Drug Interactions: Multiple human metabolizing enzymes are involved in the Phase I metabolism of MLN0128. When normalized for human liver content, the CYP isoforms CYP3A4, CYP2C9, and CYP2C19 appear to contribute to MLN0128 metabolism. MLN0128 displayed low potential (IC_{50} > 25 μM) for inhibition of the major human CYP isoforms.

Please refer to the MLN0128 Investigator's Brochure for more information.

Availability: MLN0128 is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

MLN0128 is provided to the NCI under a Collaborative Agreement between the Millennium Pharmaceuticals, Inc. and the DCTD, NCI (see Section 12.3).

8.1.1 **Agent Ordering and Agent Accountability**

8.1.1.1 NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application (https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://eapps-ctep.nci.nih.gov/iam/) and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

8.1.1.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.2 Commercial Agent: Bevacizumab

Other Names:	rhuMAb VEGF, Avastin®
Classification:	Recombinant humanized monoclonal antibody
Molecular Weight:	Approximate molecular weight is 149,000 daltons
Mode of Action:	Bevacizumab blocks the binding of vascular endothelial growth factor (VEGF) to its receptors resulting in inhibition of angiogenesis.
Description:	Bevacizumab is a recombinant humanized anti-VEGF monoclonal antibody consisting of 93% human and 7% murine amino acid sequences. The agent is composed of human IgG framework and murine antigen-binding

complementarity-determining regions

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

Preparation: Vials contain no preservatives and are intended for single use only. Place the calculated dose in 100 mL of 0.9% sodium chloride for injection.

Storage: Upon receipt, refrigerate bevacizumab (2˚ to 8˚ C). Do not freeze. Do not shake.

Stability: Shelf-life studies of rhuMAb VEGF are ongoing. The sterile single use vials contain no antibacterial preservatives. Discard vials 8 hours after initial entry.

Once diluted in 0.9% sodium chloride, administer solutions of bevacizumab within 8 hours.

Route of Administration: Intravenous

Method of Administration: Administer the initial dose over a minimum of 90 minutes. If no adverse reactions occur, administer the second dose over a minimum of 60 minutes. If no adverse reactions occur after the second dose, administer subsequent doses over a minimum of 30 minutes. If infusion-related adverse reactions occur, all subsequent infusions should be administered over the shortest period that was well tolerated.

To insure complete delivery of bevacizumab, flush the IV infusion line with 0.9% sodium chloride. The following are two recommended methods for flushing the bevacizumab IV infusion line:

1. When the bevacizumab infusion is complete, add an additional 50 mL of 0.9% sodium chloride for injection to the bevacizumab infusion bag. Continue the infusion until a volume equal to that of the volume contained in the tubing has been administered.

2. Replace the empty bevacizumab infusion bag with a 50 mL bag of 0.9% sodium chloride for injection and

infuse a volume equal to the volume contained in the tubing.

Please note: the flush is not included in the total recommended infusion times.

Agent Ordering: Bevacizumab is commercially available. The investigator or designated study personnel are responsible for maintaining dispensing records of bevacizumab per institutional standards.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

All biomarker and correlative studies will be performed only in patients participating in stage 2 or the dose expansion cohort of the study. These studies will be exploratory.

Table 9.1 Synopsis of Correlative Studies

Purpose	**Technique**	**Participants**	**Timing of collection**	**Collection requirements**	**When requested by DFCI Coordinating Center, ship to:**
Blood PK studies	Mass spectrometry on whole blood	Recurrent GBM	C1D15 and C2D1: 2-3 hours post MLN0128 dosing, prior to bevacizumab dose	3 mL blood into a plastic plasma collection tube with spray coated sodium heparin	Frozen at -70C and shipped overnight on dry ice: Jeffrey Supko, MD
CSF PK studies	Mass spectrometry on CSF	Recurrent GBM	C1D15 and C2D1: 2-3 hours post MLN0128 dosing, prior to bevacizumab dose	2 mL of CSF into a cryotube	Frozen at -70C and shipped overnight on dry ice: Jeffrey Supko, MD
Biomarker Studies on Archival Tissue	IHC on FFPE	Recurrent GBM	Archival Tissue	30 unstained slides of standard (4 or 5 micrometer) thickness	Keith Ligon, MD, PhD

				from archival tissue	
Tissue Biopsy	Radiological guided core needle biopsy. RPPA analysis and genomics studies	Ovarian and endometrial expansion cohorts	Before MLN 0128 therapy, on C1D15 prior to bevacizumab infusion and optional biopsy to be done at time of progression.	As indicated below	Frozen at -70C and shipped on dry ice to John L. Hays MD, PhD
Research blood draw for cytokines	Illumna Multiplex assay on plasma and genomics studies	Ovarian and endometrial expansion cohorts	Before MLN0128 therapy and on C1D15 prior to bevacizumab infusion, C2D1 and on the first day of each subsequent cycle	7 mL blood into a plastic plasma collection tube with spray coated sodium heparin	Shipped at 4C to John L. Hays MD, PhD

9.1 Correlative Studies for Stage 2

9.1.1 Plasma and CSF MLN0128 concentrations

9.1.1.1 Collection of the Specimens:

The plasma and CSF concentrations of MLN0128 will be characterized in all patients with recurrent GBM enrolled in Stage 2 of the study. Blood and CSF samples will be collected 2 hours after the dose is taken by the patient, on cycle 1, day 15 and cycle 2, day 1.

9.1.1.2 Handling of the Specimens:

For all pharmacokinetic plasma samples, draw 3 mL of blood into a plastic plasma collection tube with spray-coated sodium heparin at each of the time points specified above. Promptly mix the specimen by gently inverting the collection tube several times and then place it on wet ice. Centrifuge the sample at 1,100-1,300 x g for 10 min at 4°C within 30 min after collection. Remove the plasma from the blood cells using a pipette and distribute it in equal volumes into two self-standing

polypropylene cryogenic tubes with external threads. Affix a computer-printed label (protocol no., patient no., sample no., scheduled sample time) to each cryotube. Completely cover the label with protective cryogenic freezer tape (Fisher catalog no. 11-867B). Place the tubes on crushed dry ice until stored in a freezer maintained at -70°C.

For all pharmacokinetic CSF samples, collect 2 mL of CSF (obtained by performing lumbar puncture under provisions that the treating physician considers to be safe) in Nunc cryotubes. CSF studies will be omitted in participants with an increased risk of herniation. In those participants who have developed new or worsening neurologic signs and symptoms concerning for raised intracranial pressure, repeat head imaging in the form of head CT or brain MRI is recommended prior to lumbar puncture to rule out herniation. Affix a computer-printed label (protocol no., patient no., sample no., scheduled sample time) to each cryotube. Completely cover the label with protective cryogenic freezer tape (Fisher catalog no. 11-867B). Place the tubes on crushed dry ice until stored in a freezer maintained at -70°C.

The concentration of MLN0128 in study samples will be determined by using an LC-MS/MS assay. The analytical method will be validated and applied to the analysis of study samples as recommended by the FDA Guidance for Industry: Bioanalytical Method Validation, May 2001 (http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf).

9.1.1.3 Specimen Packaging and Shipping:

Send complete sets of samples from each patient (pharmacokinetic plasma, pharmacokinetic CSF) by overnight courier to the address listed below. Place the sample tubes within a zip lock plastic bag. Fill a seamless Styrofoam container with at least 3 inches of dry ice, place the plastic bag containing the samples on top of the dry ice, and completely cover the bag with an additional 3 inches or more of dry-ice. Seal the Styrofoam container within a tight-fitting cardboard shipping box. Seal a copy of the sample collection time form for each set of samples within a zip-lock plastic bag and place the bag on top of the styrofoam container before the external shipping box is sealed. Send the samples on a Monday, Tuesday, or Wednesday by overnight courier for delivery by 10:00 AM on the following day. Notification of the delivery must be made by e-mail to both Dr. Jeffrey G. Supko (email: jsupko@partners.org) and Sarah Hilderbrand (e-mail: slhilderbrand@partners.org). Include the name of the courier and the tracking no. in the email.

Dr. Jeffrey G. Supko
Massachusetts General Hospital
55 Fruit St., GRJ 1025
Boston, MA 02114
Tel: 617-724-1970

9.1.2 **Archival Tumor Samples for biomarkers**

9.1.2.1 Collection and Handling of the Specimens:

Archived tumor tissue from a prior resection of glioblastoma (pre-treatment, most recent surgery preferred but any other prior surgery tissue if most recent is not available) will be collected on all recurrent GBM patients enrolled in Stage 2 of the study. At the time of registration, prior to beginning treatment, a tumor tissue form indicating availability will need to be completed and signed by a pathologist. This form provides written documentation of the availability of tissue for this study and the pathologist's agreement to send it as described below. There will be central review of pathology by Dr. Keith Ligon at Dana-Farber Cancer Institute. The amount of tissue required for this study and registration of patients will be 30 unstained slides (standard 4-5um, no heat treatment) from the initial resection from a block selected by the local pathologist to contain >70% tumor nuclei and <10% necrosis. Size of tumor tissue should be approximately 0.5cm in diameter or greater.

The associated histopathology report as well as **ALL** ancillary testing reports for molecular or IHC studies (e.g. MGMT status, IDH1(R132H) mutation status, sequencing studies, and copy number analyses by FISH, arrays, or other methods) from the institution of collection should be shipped with the slides.

9.1.2.2 Shipping of the Specimens:

Ship archival tumor tissue and associated report(s) to Dr. Ligon below. Notification of the delivery must be made by e-mail to Dr. Keith Ligon (email: Keith_Ligon@dfci.harvard.edu). Include the name of the courier and the tracking no. in the email.

Keith L. Ligon, MD, PhD
ATTN: Adult Neuropathology CRC
Center for Molecular Oncologic Pathology
Dana-Farber Cancer Institute
450 Brookline Ave, JF215
Boston, MA 02215
Phone: 1-617-582-8764
Fax: 1-617-582-8761
Email: Keith_Ligon@DFCI.harvard.edu

9.1.3 **Endometrial and ovarian cancer patient tumor biopsies**

9.1.3.1 Collection, Handling and Shipping of Specimens:

At least three core biopsies, not less than 18-gauge in diameter and at least 1cm in length will be obtained. Inability to get tissue with a reasonable attempt will not preclude treatment and the patient will remain eligible for all other translational

components, including imaging. The use of imaging to facilitate biopsies will be decided upon by members of the interventional radiology team. We have optimized an SOP for reliable acquisition of tumor biopsies and quantification of cellular proteins and have recently reported the success and safety of obtaining serial biopsies in patient on clinical trials[63].

Upon acquisition, biopsy cores will be snap frozen in OCT on dry ice according to SOP in Appendix D and samples will mailed on dry ice to Dr. John Hays. Notification of the delivery must be made by e-mail to Dr. Hays (john.hays@osumc.edu) and please include the name of the shipping company and the tracking number in the e-mail.

For all research blood samples, draw 7 mL of blood into a plastic green top (Heparin containing) vacutainer at each of the time points specified in the research calendar. Promptly mix the specimen by gently inverting the collection tube several times and then place it on wet ice. Centrifuge the sample at 1,100-1,300 x g for 10 min at 4°C within 30 min after collection. Remove the plasma from the blood cells using a pipette and distribute it in equal volumes into 6 self-standing polypropylene cryogenic tubes (Sigma, Cat #V5007) with external threads. Remove the buffy coat layer and place into 1 self-standing polypropylene cryogenic tubes with external threads (Sigma, Cat #V5007). Affix a computer-printed label (protocol no., patient no., sample no., scheduled sample time) to each cryotube. Completely cover the label with protective cryogenic freezer tape (Fisher catalog no. 11-867). Place the tubes on crushed dry ice until stored in a freezer maintained at -70°C until shipment.

John L. Hays MD, PhD
Biomedical Research Tower, Rm 560
460 W. 12th Avenue,
Columbus, OH 43210

9.1.3.2 Genomics Analysis

Mutational analysis and sequencing will be performed on the first biopsy specimen and research blood draw (prior to therapy) and an optional third biopsy specimen at progression of disease. We will employ clinical sequencing in for a custom cancer panel of 220 genes in a CLIA certified lab (Roychowdhury Lab). We have previously demonstrated feasibility for utilizing small tumor biopsy specimens and have a 95% success rate[64]. The custom cancer panel includes assessment of both DNA and RNA for 220 oncogenes and tumor suppressors (including PTEN, AKT1, KRAS, BRAF, FLT3, SRC, TP53, EGFR, NRAS, and PIK3CA) and is designed to detect point mutations, copy number changes, and gene fusions. Targeted gene sequencing is accomplished by solution-based hybridization capture with custom baits (Agilent) of both tumor (biopsy) and normal DNA (buffy coat isolate from blood obtained for circulating cytokines). This is used to generate somatic point mutations and copy number changes using published tools (Varscan2, MuTect). Targeted gene sequencing from RNAseq libraries is utilized to identify gene fusions and analyzed using

published fusion tools (Tophat2, Chimerscan). All sample processing sequencing, and analysis will be completed in Dr. Roychowdhury's lab and are standardized locked-down procedures.

9.1.3.3 Proteomics Analysis

Reverse phase protein arrays will be performed on patient biopsies to monitor the amount and phosphorylation states of the following proteins: MEK1/2 (S217/221), AKT (S473, T308), PI3K, p70S6K (T389), MAPK1/2 (T202/204), GSK3beta(S21/S9), p27 (T157, T198), p38MAPK (T180/182), rS6K (S235/236, S240/244), and 4E-BP1 (S65). These proteins represent a sample across multiple canonical pathways that have demonstrated importance in VEGF and PI3K mediated signaling in various cancers[7,65] and tumor microenvironment interactions[66]. RPPA assesses the qualitative and quantitative changes in defined endpoints using a microarray platform[67]. Clinical samples will be sliced and then lysed with total protein quantitated using BCA assay (Thermo Scientific, Rockford, IL). Sample analysis will take place at the MD Anderson Proteomics Core Facility. Samples will serially diluted and then arrayed on slides using an Auschon 2470 Arrayer (Auschon Biosystems, Billerica, MA). Each slide will be probed with a titer optimized primary antibody as we have done previously with clinical samples[67]. The signal will be analyzed using a DAKO CSA tyramide-amplification approach and visualized by DAB colorimetric reaction [68]. Spots will be identified and their densities quantified by MicroVigene. Relative protein levels for each sample will be determined by interpolation of each dilution curve from the "standard curve" of the slide/antibody (MD Anderson Bioinformatics core facility). Our studies have previously demonstrated that RPPAs provide an efficient method for quantifying protein expression of multiple protein endpoints from the same sample. Previous results in our laboratory have shown >30 printings per sample is achievable with good reproducibility[67]. Please refer to Appendix F for details regarding reverse phase protein arrays.

9.1.3.4 Circulating Cytokines

Blood samples will also be obtained as described above (in section 9.1.1.2) to measure levels of circulating cytokines by standard ELISA. Levels of VEGFA, PIGF, bFGF , VEGFD, MMP2, MMP9, IL6, IL8, EGF and angiopoietin will be determined at the specified time points. Briefly, we will be using a multiplexed bead based array system (EMD Millipore with R&D Systems multiplexed performance assays bead panels). Cytokine selection is based upon published reports of proteins and growth factors believed to be important in vascular formation (VEGFA,VEGFD, IL6, IL8, Angiopoietin-1, bFGF), tumor aggressiveness and invasion (IL-6, IL-8, EGF, MMP2, MMP9), resistance/sensitivity to bevacizumab mediated therapy (VEGFA, VEGFD, IL-6, Angiopoietin-1), or linked with signaling through PI3K/AKT/mTOR pathway. Seven milliliters of blood will be collected in a heparinized tube (BD Green top) for cytokine analysis and buffycoat isolation. Sample will be drawn and stored on ice until centrifugation. Samples will be centrifuged within 60 minutes of collection. Using a Pasteur pipette, plasma will be collected and placed in 5ml Nunc tube, labeled

with subject ID and collection time, and stored at -70 degrees until shipment or analysis. Using a fresh Pasteur pipette, transfer buffy coat into 2ml Nunc tube, label with patient ID and collection time, and store at -70 degrees until shipment or analysis. The analytes can be covered with three different multiplexed arrays, each needing <100 microliters of plasma.

9.1.3.5 Apoptosis Assay

Apoptosis assays will be performed with the validated triplex Rx1-IFA4 assay (γH2AX, Ki67, cleaved caspase 3, DAPI), through the DCTD laboratories after appropriate training of the PI through the DCTD

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 14 days of registration. GBM participants must have baseline scan performed within 14 days of registrations; scans for all other patients must be done ≤4 weeks prior to registration. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

Assessments	Screening [a]	Cycle 1				Cycle 2				Subsequent Cycles		End of Tx [e]	30-day post drug [f]	Long Term Follow Up [g]
		D1[b]	D8[c]	D15[c]	D22[c]	D1[d]	D8[c]	D15[c]	D22[c]	D1[d]	D15[c]			
Informed consent [h]	X													
Background information/history [i]	X													
Inclusion/exclusion criteria [j]	X													
Vital signs [k]	X	X	X	X	X	X		X		X	X	X		
Physical exam [l]	X	X	X	X	X	X		X		X	X	X		
Neurologic Exam [m]	X	X	X	X	X	X		X		X	X	X		
Performance Status [n]	X	X	X	X	X	X		X		X	X	X	X	
Concomitant medications [o]	X [p]	----------X [o]----------												
Adverse event assessment [q]		----------X [q]----------												
12-lead ECG [r]	X	X [r]				X [r]				X [r]		X		
MUGA or ECHO	X													
Imaging – CT or MRI [s]	X									X [t]		X		
Response assessment [t]										X [t]		X		
Hematology [u]	X	X	X	X	X	X	X	X	X	X	X	X		
Serum Chemistry [v]	X	X	X	X	X	X	X	X	X	X	X	X		
Fasting serum glucose [w]	X	X	X	X	X	X	X	X	X	X	X	X		
HbA1c [ff]	X									X [ff]				
Fasting lipid profile [w]	X	X				X				X		X		
Coagulation PT/INR- [x]	X			X		X						X [ee]		
Urinalysis [y]	X	X		X		X				X		X		
Serum Pregnancy Test [z]	X					X				X		X		
MLN0128 [aa]		----------X [cc]----------												
Bevacizumab [bb]		X [bb]		X [bb]		X [bb]		X [bb]		X [bb]	X [bb]			
Blood PK [cc]				X		X								
CSF PK [cc]				X		X								
Biopsy [cc]		X		X								X		
Blood for cytokines [cc]		X		X		X				X				
Submission of archival tissue [ff]		X [ff]												
Survival [g]													X	X

a. All screening procedures to be performed within 14 days of registration, except imaging within 14 days from start of study treatment for GBM participants and within 28 days from start of study treatment for all other participants.

b. Cycle 1, Day 1 assessments to be performed within 2 days prior to MLN0128

c. Mid cycle assessments have a +/- 2 days window.

d. Within 2 days (48 hours) prior to Day 1 drug, except imaging which are within 7 days prior to Day 1 drug.

e. End of treatment assessments to be performed within 7 days after last study drug or within 7 days after decision to end treatment.

f. A contact/visit for review of adverse events, KPS, and vital status is to be performed at 30 days (+/- 5 days) after the last study drug is given (may be performed by documented clinician telephone call, if visit is not feasible). This may be performed via documented phone conversation with a study nurse or clinician. Participants with ongoing reportable adverse events may require additional follow-up per section 11.1.

g. Long-term Follow-Up: Per Section 5.6, participants will be following annually for survival via telephone or medical record following until patient either passes away or is lost to long-term follow-up. Progression information will be collected for participants who do not progress while on study treatment.

h. Informed Consent: performed by MD attending only. Informed consent process to be fully documented: e.g. prospective participant had sufficient time for deliberation, all questions were answered, treatment options provided by MD, full study reviewed including risks, and a copy of signed and dated consent given to participant.

i. Background/history: to review treatment history for treated cancer, any ongoing medical conditions and medical history pertaining to eligibility on study and involvement during study.

j. Inclusion/exclusion criteria: source documentation providing Investigator's confirmation that patient has met all eligibility criteria must be available prior to registration.

k. Vital signs: weight, heart rate, blood pressure, respiration rate, temp, oxygen saturation. Height required only at baseline.

l. Physical Exam: physical exam.

m. Neurologic exam required for recurrent GBM participants only.

n. For all non-GBM participants, the performance status will be assessed according to the ECOG (WHO) performance status scale. For all GBM participants, the performance status will be assessed according to the Karnofsky Performance Status (KPS) scale. See Appendix A for scales.

o. Concomitant medications and reason for administration should be documented in the case history. All medications taken within 30 days of screening up until 30-day post drug visit should be recorded

p. At baseline/screening, specific start dates are requested for steroids, AEDs, anti-coagulants, and drugs for hypertension. For all other concomitant medications at baseline/screening, knowledge of whether or not agent began within last thirty days will be requested for the eDC.

q. Adverse events (AEs) experienced by participants will be collected and recorded from first dose of study drug up to 30 days of the last dose of study medication. The period for collection and recording of AEs is extended for participants with ongoing reportable adverse events that are related to study agent (see Section 7.1). Adverse event reporting begins for events that occur after registration.

r. ECG: C1D1 pre-dose, C1D1 2 hour post MLN0128 dose (+/- 10 minutes), C1D1 4 hours post MLN0128 dose (+/- 10 minutes); C2D1 pre-dose, C2D1 2 hours post MLN0128 dose (+/-10 minutes); subsequent day 1 ECGs to be done 2 hours post MLN0128 dose (+/- 10 minutes).

s. CT with or without contrast or MRI with and without contrast.

t. Within 7 days prior to day 1 of odd cycles (3, 5, 7, etc.).

u. Hematology – erythrocytes (RBC), hemoglobin, hematocrit, platelets, total WBC plus absolute differential counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils).

v. Chemistry - albumin, alkaline phosphatase (ALP), bicarbonate (HCO3), BUN, calcium, chloride, creatinine, magnesium,(GBM patients only), phosphorous, LDH, potassium, SGOT (AST), SGPT (ALT), sodium, total protein, and total bilirubin. If total bilirubin is greater than the upper limit of normal, direct and indirect bilirubin should be performed.

w. Fasting lipid profile: total cholesterol, HDL-C, LDL-C, triglycerides (8 hours fasting).

x. Coagulation: PT/INR,

y. Urinalysis: specific gravity, pH, glucose, protein, bilirubin, ketones, leukocytes, and blood. Per Table 6.1, if protein is ≥ 2+, obtain 24-hour urine protein.

z. Serum pregnancy test only for women of child bearing potential.

aa. MLN0128 to be taken in clinic on days of scheduled pharmacokinetic sampling and pre/post MLN0128 dose ECGs.

bb. Bevacizumab to be administered on C1D1 on days 1 and 15 (+/- 2 days) for each 28-day cycle, except for participants enrolled in Stage 2 who will not receive bevaizumab on C1D1 and will start treatment with bevacizumab on C1D15. Please refer to Section 5.1.2 for timing of bevacizumab infusions.

cc. All biomarker and correlative studies will be performed only in participants enrolled in the dose expansion cohort of stage 2 of this study. Please refer to Section 9 for more details.

dd. Submission of archival tissue required for GBM participants to be submitted within 60 days after registration. See Section 9.1.2 for details.

ee. Coagulation tests to be done at end of treatment visit only for participants undergoing biopsy.

ff. HbA1c is required at screening and prior to initiation of odd cycles (C3D1, C5D1, etc) only.

11. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated every 8 weeks. In addition to a baseline scan, confirmatory scans will also be obtained 8 weeks following initial documentation of an objective response.

11.1 Antitumor Effect – Non-GBM Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 8 (not less than 4) weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)[60]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with MLN0128.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray or as ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not

be considered measurable.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

11.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the

treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one

assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers Tumor markers alone cannot be used to assess response.

Cytology, Histology These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. Due to the complexity of interpreting FDG-PET results with mTORC1/2 inhibitors, FDG-PET will not be used to upgrade a response to CR.

Note: A ‘positive’ FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.6 Progression-Free Survival

From date of first dose to date of progression or death. Participants who stop treatment for causes other than progression may be censored if other therapy is initiated or if regular assessments for assessing progression are no longer available.

11.2 Antitumor Effect – Glioblastoma patients

This trial will utilize the criteria recently proposed by the Response Assessment in Neuro-Oncology (RANO) working group[61]. The RANO Criteria updates its established predecessor, the Macdonald Criteria.

11.2.1 Antitumor Effect – Definitions

Evaluable for toxicity. All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for objective response. Only those participants who have measurable disease present at baseline and have received at least one cycle of study therapy will be considered evaluable for response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression or die prior to the end of cycle 1 will also be considered evaluable.)

Measurable disease. Bidimensionally, contrast-enhancing, measurable lesions with clearly defined margins by CT or MRI scan, with a minimal diameter of 1 cm, and visible on 2 axial slices which are at least 5 mm apart with 0 mm skip. Measurement of tumor around a cyst or surgical cavity, if necessary, requires a minimum thickness of 3 mm. If there are too many measurable lesions to measure at each evaluation, the investigator must choose the largest two to be followed before a participant is entered on study. The remaining lesions will be considered non-measureable for the purpose of objective response determination. Unless progression is observed, objective response can only be determined when all measurable and non-measurable lesions are assessed.

Non-measurable evaluable disease. Unidimensionally measurable lesions, masses with margins not clearly defined, lesions with maximal diameter < 1cm.

11.2.2 Response/Progression Categories

Complete response (CR).

All of the following criteria must be met:

a. Complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks. In the absence of a confirming scan 4 weeks later, this scan will be considered only stable disease.
b. No new lesions.
c. All measurable and non-measurable lesions must be assessed using the same techniques as baseline.
d. Participants must be on no steroids or on physiologic replacement doses only.
e. Stable or improved non-enhancing (T2/FLAIR) lesions
f. Stable or improved clinically, for clinical signs and symptoms present at baseline and recorded to be disease related.
g. Participants with non-measurable disease cannot have a complete response. The best response possible is stable disease.

Partial response (PR).

All of the following criteria must be met:

a. Greater than or equal to 50% decrease compared to baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks. In the absence of a confirming scan 4 weeks later, this scan will be considered only stable disease.
b. No progression of non-measurable disease.
c. No new lesions.
d. All measurable and non-measurable lesions must be assessed using the same techniques as baseline.
e. The steroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan.
f. Stable or improved non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared to baseline scan.
g. Stable or improved, for clinical signs and symptoms present at baseline and recorded to be disease related clinically.

Participants with non-measurable disease cannot have a partial response. The best response possible is stable disease.

Progressive disease (PD).

The following criterion must be met:

a. > 25% increase in sum of the products of perpendicular diameters of enhancing lesions (over best response or baseline if no decrease) on stable or increasing doses of corticosteroids and/or one or more of the of the following:
b. Significant increase in T2/FLAIR non-enhancing lesion on stable or increasing doses of corticosteroids steroids compared to baseline scan or best response following initiation of therapy, not due to co-morbid events (radiation therapy, demyelination, ischemic injury, infection, seizures, post-operative changes, or other treatment effects).
c. Any new lesion

d. Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication side effects, complications of therapy, cerebrovascular events, infection, etc.). The definition of clinical deterioration is left to the discretion of the investigator but it is recommended that a decline in the Karnofsky Performance Score (KPS) from 100 or 90 to 70 or less, a decline in KPS of at least 20 from 80 or less, or a decline in KPS from any baseline to 50 or less, for at least 7 days, be considered neurologic deterioration, unless attributable to co-morbid events or changes in corticosteroid dose.
e. Failure to return for evaluation due to death or deteriorating condition

Stable disease (SD).
All of the following criteria must be met:
a. Does not qualify for CR, PR, or progression.
b. All measurable and non-measurable sites must be assessed using the same techniques as baseline.
c. Stable non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared to baseline scan. In the event that the corticosteroid dose has been increased, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.
d. Stable clinically.

Unknown response status.
Progressive disease has not been documented and one or more measurable or non-measurable lesions have not been assessed.

Table 10.2 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

11.2.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation, using a ruler, calipers, or digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and ideally within 14 days before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

11.2.4 Evaluation of Best Response

The best overall response is the best response recorded from the start of the post-surgical treatment until disease progression (taking as reference for progressive disease the smallest measurements recorded since the treatment started). If a response recorded at one scheduled MRI does not persist at the next regular scheduled MRI, the response will still be recorded based on the prior scan, but will be designated as a non-sustained response. If the response is sustained, i.e. still present on the subsequent MRI, it will be recorded as a sustained response, lasting until the time of tumor progression. Participants without measurable disease may only achieve SD or PD as their best "response."

11.2.5 Other Effect Measures

Neurological Exam
Although not used for determining response, it is useful to evaluate changes in the neurological exam compared to the previous exam. The following scale may be used:

+2	Definitely better
+1	Possibly better
0	Unchanged
-1	Possibly worse
-2	Definitely worse

Performance Status
Participants will be graded according to KPS score

Overall survival time
From date of first dose to date of death due to any cause.

Progression-free survival time
From date of first dose to date of progression or death. Participants who stop treatment for causes other than progression may be censored if other therapy is initiated or if regular assessments for assessing progression are no longer available.

11.2.6 Response Review

Central review of MRI or CT scans is planned for participants who achieve CR, PR, or PFS6.

Central review will be performed at Brigham and Women's Hospital on registered participants who have been determined by the enrolling institution as having achieved PFS6, complete radiographic response, or partial radiographic response. When a participant's films are requested, all films of all views from pre-registration and subsequent scans must be submitted for central review. Once the Central Review is complete, the central review results will be made available to the local PI.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The QACT will collect, manage, and monitor data for this study.

This study will also be monitored by the Clinical Data Update System (CDUS). Cumulative CDUS data will be submitted quarterly to CTEP via electronic means by the Coordinating Center for all participants. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the Clinical Data Update System can be found on the CTEP website at: http://ctep.cancer.gov/protocoldevelopment.

Note: All adverse events that are reported through CTEP-AERS must also be reported via CDUS.

12.1.2 Data Submission

The schedule for completion of submission of case report forms (paper or electronic) to the QACT is as follows:

Form	Suggested Submission Timeline
Eligibility Checklist	Complete prior to registration with QACT
On Study and Baseline Forms	Within 14 days of registration
Treatment Forms	Within 10 days of the last day of the cycle
Adverse Event Report Forms	Within 10 days of the last day of the cycle
Response Assessment Forms	Within 10 days of the last day of the cycle required for response evaluation
Off Treatment/Off Study Forms	Within 14 days of completing treatment or being taken off study for any reason
Follow Up/Survival Forms	Within 14 days of the protocol defined follow up visit date or contact

Participating Institutions are responsible for submitting CDUS data and/or data forms to the

Coordinating Center on the study quarterly to allow time for Coordinating Center compilation. The date for submission to the Coordinating Center will be set by the Overall PI. CDUS does not accept data submissions from the participants on the study. When setting the dates, allow time for Coordinating Center compilation, Principal Investigator review, and timely submission to CTEP by the quarterly deadlines (see Section 12.1.1).

The Coordinating Center is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.2 CTEP Multicenter Guidelines

This protocol will adhere to the policies and requirements of the CTEP Multicenter Guidelines. The specific responsibilities of the Principal Investigator and the Coordinating Center (Study Coordinator) and the procedures for auditing are presented in Appendix B.

- The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required.

- Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov) except for Group studies.

12.3 Collaborative Agreements Language

The agent supplied by CTEP, DCTD, NCI used in this protocol is provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

General considerations:

Patients will be enrolled in this multicenter, open-label phase I study with dose escalation in a 3+3 design followed by dose confirmation in 3 tumor types. During dose escalation, patients will be enrolled in to sequential cohorts; the total number patients in a specific cohort will be determined based on the occurrence of DLTs at that dose level (refer to Section 5.1 and 5.5) with a range of 2-6 patients per cohort. During dose confirmation, 10 patients will be enrolled in each of the 3 tumor-specific cohort expansions, except in ovarian and endometrial cancer cohorts in which a maximum of 15 patients each may be enrolled.

Primary Endpoints:

- To determine the MTD/RP2D and dose limiting toxicity (DLT) of daily oral MLN0128 when administered with bevacizumab in patients with advanced solid tumors including recurrent GBM.
- To evaluate the safety and tolerability of the combination of MLN0128 and bevacizumab.

Enrollment, major protocol violations, and discontinuations from the study will be summarized by dose level.

Demographic and baseline characteristics, such as age, sex, race, weight, type of malignancy, duration of malignancy, and baseline Karnofksy Performance Status, will be summarized using means, standard deviations, medians, and ranges for continuous variables, and proportions for categorical variables. All summaries will be presented overall and by dose level. Study drug administration data will be listed by dose level, and any dose modifications will be flagged. Means and standard deviations will be used to summarize the total dose of MLN0128 and bevacizumab received.

Safety will be assessed through summaries of adverse events, changes in selected laboratory test results, changes in vital signs, and MLN0128 and bevacizumab exposure. All patients who receive any amount of study treatment will be included in the safety analysis.

For the dose escalation portion, the following table shows the probability of escalating the dose for various true, but unknown, rates of unacceptable toxicity.

True DLT Rate	Probability of Dose Escalation
10%	91%
20%	71%
30%	49%
40%	31%
50%	17%
60%	8%

13.2 Sample Size/Accrual Rate

13.2.1 Sample Size Calculation

Stage 1:

Standard cohorts of three designs will be applied. Patients will be accrued in cohorts of 3 starting at dose level 1.

The MTD will be based on the assessment of DLT during the first 4 weeks of treatment only (i.e. following the first 2 treatment cycles), and will be defined as the dose at which fewer than one-third of patients experience a DLT to study treatment. The MTD is the dose level at which 0/6 or 1/6 patients experience DLT with the next higher dose having at least 2 out of 3 or 2 out of 6 patients encountering DLT.

Three patients will be treated at each dose level, and can be enrolled simultaneously. If one DLT is encountered, an additional 3 patients will be added to that dose level. If at any point two DLTs are encountered within a given dose level, then the MTD has been exceeded and if only three patients have been treated at the next lower dose three more patients are treated at the next lower dose.

If it is determined that dose level 1 exceeds the MTD, then additional reduced dose level (-1) will be discussed. No dose levels above dose level 3 will be explored.

A minimum of 12 and a maximum of 18 patients will be accrued in this part of the study.

Stage 2:

After the MTD/RP2D is established, a maximum of 40 patients; 10 (maximum of 15) with recurrent or advanced ovarian cancer, 10 (maximum of 15) with recurrent endometrial cancer (depending on biopsies, accrual will stop as soon as 10 adequate tissue are obtained but no more than 5 failures will be covered by additional enrollment in these expansion cohorts) and 10 with recurrent GBM will be accrued. The dose expansion part is primarily conducted and sized for continued safety evaluation If 2 or more patients in each dose-expansion cohort develop

unacceptable toxicity defined as grade 4 or 5 non-hematological toxicity or ≥grade 2 intracranial hemorrhage (in recurrent GBM patients), then further accrual to that cohort will be stopped and the next dose level down will be considered the MTD for that particular cohort. If the true unacceptable toxicity rate is as low as 0.05 then the probability of observing 2 or more of the above adverse events in the expansion cohort is 0.08, while if it is as high as 0.3 then this probability is 0.85. The stopping rule will be assessed separately in each cohort.

For the ovarian and endometrial cancer cohorts, we estimate that 10 patients per arm will provide power for exploratory translational analyses examining target presence, activation, modulation, and relationship to clinical outcome. This plan will allow the discovery of possible predictive biomarkers that can be queried in future trials with this combination. To be conservative, the sample size required was calculated under the assumption that a Bonferroni adjustment is required for the 10 primary evaluations to be performed during the RPPA analysis (MEK1/2 (S217/221), AKT (S473, T308), PI3K, p70S6K (T389), MAPK1/2 (T202/204), GSK3beta(S21/S9), p27 (T157, T198), p38MAPK (T180/182), rS6K (S235/236, S240/244), and 4E-BP1 (S65)). Thus, requiring a two-tailed p-value <0.005 (=0.05/10) for each test to be considered statistically significant will be used to estimate the desired number of subjects. In practice, a Hochberg adjustment will be used to evaluate the significance of the multiple comparisons, which is not as stringent as a Bonferroni adjustment, but will still maintain an overall 0.05 significance level for the comparisons performed. Furthermore, since this study is mainly pilot in intent, the findings will be reported in that context. With 10 patients in a cohort, for each such comparison, there is 90% power to detect a difference from baseline equal to 20% change from baseline for each of the 10 parameters (assuming a mean CV of 6.7%), with an overall alpha=0.005 as stated above. This calculation assumes that a paired t-test will be used to test the difference in time for each parameter. If these changes are not normally distributed ($p<0.05$ by Shapiro-Wilks test), then a Wilcoxon signed rank test will be used instead of a t-test. In addition, a paired t-test or Wilcoxon signed rank test will be used to evaluate the significance of changes for each correlative end point.

In an exploratory fashion, we hypothesize that patients harboring tumors with activating genomic alterations in PTEN, PIK3CA and AKT1 will show increased sensitivity and tumor response to use of MLN0128. To evaluate this hypothesis we will test an expansion cohort of 10 endometrial cancer patients. A recent study in patients with recurrent endometrial cancer patients indicated 17/31 patients harbored a genetic alteration in PTEN. The actual amount of PIK3CA mutation in this cohort is unknown, but activation of AKT (by measurement of pAKT) has generally demonstrated approximately 50% of patients with activation of this pathway. 10 patients overall would provide 80% power (with an alpha = 10%) to detect a difference in progression free rate of about 50%, at three months between those harboring activating genomic alterations and those who do not using one-sided Z test with pooled variances.

13.2.2 Accrual rate:

We estimate accrual of 2 patients per month for stage 1, with target goal for completion of accrual in 6 months. We estimate accrual of 2 patients per months for each cohort of stage 2, with target goal for completion of accrual in 6 months.

Accrual of Women and Minorities:

For stage 2, patients accrued to ovarian and endometrial cancers, will be all women.

Accrual Targets								
Ethnic Category	**Sex/Gender**							
	Females			**Males**			**Total**	
Hispanic or Latino	3		+	1		=	4	
Not Hispanic or Latino	28		+	10		=	38	
Ethnic Category: Total of all subjects	31	(A1)	+	11	(B1)	=	42	(C1)
Racial Category								
American Indian or Alaskan Native	0		+	0		=	0	
Asian	1		+	1		=	2	
Black or African American	3		+	1		=	4	
Native Hawaiian or other Pacific Islander	0		+	0		=	0	
White	27		+	9		=	36	
Racial Category: Total of all subjects	31	(A2)	+	11	(B2)	=	42	(C2)
	(A1 = A2)			(B1 = B2)			(C1 = C2)	

13.3 Stratification Factors

None planned.

13.4 Analysis of Secondary and Exploratory Endpoints

13.4.1 Secondary Endpoints

To assess the preliminary anti-tumor activity the combination of MLN0128 and bevacizumab, as determined by progression-free survival (PFS6), response rate (RR) and overall survival (OS).

Response assessment data, PFS, and duration of response will be listed for all patients by dose level.

Objective response rate will be estimated for patients, with disease measurable by RECIST or RANO (for recurrent GBM) guidelines. Objective response is defined as a complete or partial response, as determined by investigator assessment using RECIST or RANO. Patients with

missing or no response assessments will be classified as non-responders. Among patients with an objective response, duration of response will be defined as the time from the initial complete or partial response to the time of disease progression or death. If a patient does not experience disease progression or death before the end of the study, duration of response will be censored at the day of the last tumor assessment.

- To assess tolerability throughout study therapy with MLN0128 and bevacizumab, including beyond the MTD interval with the following measures of cumulative treatment-related toxicities:
    - Frequency of toxicities leading to missed doses or delays
    - Percentage of cycles given or not within 7 days of their scheduled times
    - Percentage of actual planned dosage administration
    - Percentage of patients that discontinue study drugs due to treatment related toxicity.

The above percentages will be summarized.

13.4.2 Exploratory Endpoints

- To assess CSF penetration of MLN0128 in combination with bevacizumab in patients with recurrent GBM by evaluating the plasma and CSF pharmacokinetic profile of MLN0128 in the absence and presence of bevacizumab.
- To perform archival tumor analysis for markers associated with dysregulated cell signaling that may predict response to mTOR inhibitor therapy such as EGFR (expression by IHC and amplification by FISH), PTEN (expression by IHC and deletion by FISH), p-AKT, p-S6K, p-4EBP, p-mTOR and p-Erk in patients with recurrent GBM.

Plasma and CSF PK levels of MLN0128 obtained before and after bevacizumab administration will be evaluated and summarized. The ratio of plasma to CSF PK levels will also be summarized.

The biomarkers predicting response to mTOR inhibitor activity will be resulted by dose level and response status.

- Analysis of phosphorylated proteins (MEK1/2 (S217/221), AKT (S473, T308), PI3K, p70S6K (T389), MAPK1/2 (T202/204), GSK3beta(S21/S9), p27 (T157, T198), p38MAPK (T180/182), rS6K (S235/236, S240/244), and 4E-BP1 (S65)) at baseline and the change in these proteins after treatment with MLN0128 within tumor biopsies from patients with ovarian and endometrial cancers.
- Analysis of circulating plasma levels of angiogenic growth factors before, during and after treatment with MLN0128 and bevacizumab in patients with ovarian and endometrial cancers.
- Mutation analysis of tissue from biopsies of patients with ovarian and endometrial cancers including analysis of KRAS, BRAF, PIK3CA, AKT1 and PTEN.

Please refer to section 13.2.1

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B CTEP MULTICENTER GUIDELINES

If an institution wishes to collaborate with other participating institutions in performing a CTEP sponsored research protocol, then the following guidelines must be followed.

Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

Inclusion of Multicenter Guidelines in the Protocol

- The protocol must include the following minimum information:
  - The title page must include the name and address of each participating institution and the name, telephone number and e-mail address of the responsible investigator at each participating institution.
  - The Coordinating Center must be designated on the title page.
  - Central registration of patients is required. The procedures for registration must be stated in the protocol.
  - Data collection forms should be of a common format. Sample forms should be submitted with the protocol. The frequency and timing of data submission forms to the Coordinating Center should be stated.
  - Describe how AEs will be reported from the participating institutions, either directly to CTEP or through the Coordinating Center.
  - Describe how Safety Reports and Action Letters from CTEP will be distributed to participating institutions.

Agent Ordering

- Except in very unusual circumstances, each participating institution will order DCTD-supplied investigational agents directly from CTEP. Investigational agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.

APPENDIX C STRONG INHIBITORS AND STRONG INDUCERS OF CYP2C9, CYP2C19 AND CYPE3A4

Strong Inhibitors	Strong Inducers
indinavir	carbamazepine
nelfinavir	phenobarbital
ritonavir	phenytoin
clarithromycin	rifabutin
itraconazole	St. John's wort
ketoconazole	troglitazone
nefazodone	secobarbital
fluconazole	rifampin
saquinavir	
telithromycin	
fluvoxamine	
telithromycin	
fluvoxamine	
mibefradil	
omeprazole	
Ticlopidine	

Fruits and juice
Star fruit and juice
pomegranate fruit and juice
grapefruit and juice
Seville oranges and juice
papaya fruit and juice

Sources: ganfyd.org/index.php?title=Inhibitors_of_CYP3A4 and medicine.iupui.edu/clinpharm/ddis/

APPENDIX D DATA AND SAFETY MONITORING PLAN

DFCI IRB Protocol #*:* 14-079

Dana-Farber/Harvard Cancer Center
Multi-Center Data and Safety Monitoring Plan

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1.0 INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP should serve as a reference for any sites external to DF/HCC that will be participating in the research protocol.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Standard Operating Procedures, and Cancer Therapy Evaluation Program (CTEP) Multi-Center Guidelines.

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

DF/HCC Multi-center Protocol: A research protocol in which one or more outside institutions are collaborating with Dana-Farber/Harvard Cancer Center where a DF/HCC investigator is the sponsor. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: One of the Dana-Farber/Harvard Cancer Center consortium members (Dana-Farber Cancer Institute (DFCI), Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIDMC), Children's Hospital Boston (CHB), Brigham and Women's Hospital (BWH)) responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (CTEP, Food and Drug Administration (FDA), etc.). The Lead Institution is typically the home of the Protocol Chair. The Lead Institution also typically serves as the Coordinating Center for the DF/HCC Multi-Center Protocol.

Protocol Chair: The person sponsoring the submitted Multi-Center protocol. Within DF/HCC, this person is the Overall Principal Investigator who takes responsibility for initiation, management and conduct of the protocol at all research locations. In this protocol, the Protocol Chair will serve as the single liaison with any regulatory agencies (i.e. CTEP Protocol and Information Office (PIO), etc.). The Protocol Chair has ultimate authority over the protocol and is responsible for the conduct of the study at DF/HCC and all Participating Institutions. In this case the Protocol Chair is the same person as the DF/HCC Principal Investigator; however, both roles can be filled by two different people.

Participating Institution: An institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC Investigator. The Participating Institution acknowledges the Protocol Chair as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: The entity (i.e. Lead Institution, Medical Monitor, Contract Research Organization (CRO), etc) that provides administrative support to the Protocol Chair in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines (i.e. CTEP Multi-Center Guidelines). In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol. Should the Protocol Chair decide to use a CRO, the CRO will be deemed the Coordinating Center.

DF/HCC Quality Assurance Office for Clinical Trials: A unit within DF/HCC developed to computerize and manage data, and to provide a Quality Control and Quality Assurance function for DF/HCC trials.

2.0 GENERAL ROLES AND RESPONSIBILITIES

In accordance with CTEP Multi-Center Guidelines, the Protocol Chair, Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

2.1 Protocol Chair

The Protocol Chair, Lakshmi Nayak, MD, will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Submit the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Assure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team receives adequate protocol training and/or a Site Initiation Visit prior to enrolling participants and throughout trial's conduct as needed.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC and other applicable (i.e. CTEP, FDA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with CTEP/PIO Office (CTEP trials).
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the Protocol Chair.
- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.

2.2 Coordinating Center

The Coordinating Center will assume the following general responsibilities:

- Assist in protocol development
- Maintain copies of Federal Wide Assurance and Institutional Review Board (IRB) approvals from all Participating Institutions.
- Maintain CTEP correspondence.
- Maintain updated roster of participants.
- Verify eligibility.
- Verify response.
- Collect data on protocol specific CRFs.
- Maintain documentation of Serious Adverse Event (SAE) reports submitted by Participating Institutions to CTEP.
- Provide Participating Institutions with information regarding DF/HCC requirements that they will be expected to comply with.
- Monitor Participating Institutions either by on-site or virtual monitoring.

- Maintain Regulatory documents of all Participating Institutions.
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc).
- Maintain documentation of all communications.
- Ensure that each Participating Institution has the appropriate assurance on file with the Office of Human Research Protection (OHRP).
- Compile and submit a complete report via the Clinical Data Update System (CDUS) quarterly for all participants.

2.2.1 Coordinating Center Contact Information

The DF/HCC Coordinating Center will consist of designees from both the Neuro-Oncology Department, as well as the Early Drug Development Center (Phase I). Responsibilities will be split within the departments as specified below, although may be updated based on Protocol Chair discretion.

- Central email addresses to be used for general site questions: dfcieddc_regulatory@dfci.harvard.edu, and NeuroOnc_Coor@dfci.harvard.edu
- EDDC: Regulatory functions including but not limited to amendments, teleconferences, INDSR and SAE review and circulation, overall coordination of accrual, etc.
    - Study Coordinator: Ketki Bhushan
        - Phone: (617) 632-4270
        - Email: kbhushan@partners.org
- Neuro-Onc: Monitoring functions including but not limited to site initiation visits, monitoring plan development and maintenance, eligibility review and registration, interim monitoring visits, violation tracking, etc.
    - Responsible Data Manager (Monitor): Katrina Smith
        - Phone: (617) 632-3780
        - Email: khsmith@partners.org

2.3 DF/HCC Quality Assurance Office for Clinical Trials (QACT)

In addition to the Coordinating Center, the DF/HCC QACT provides the following support services to assist the Protocol Chair:

- Develop protocol specific case report forms (CRF/eCRFS).
- QA/QC data of protocol specific CRFs.
- Provide a central participant registration, which includes review of consent and eligibility.
- Provide auditing services (funding and QACT approval required).

2.4 Participating Institution

Each Participating Institution is expected to comply with all applicable Federal Regulations and DF/HCC requirements, the protocol and HIPAA requirements. All Participating Institutions will provide a list of personnel assigned to the role for oversight of data management at their site to the Coordinating Center.

The general responsibilities for each Participating Institution are as follows:

- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.

- Maintain a regulatory binder in accordance with DF/HCC requirements.
- Provide the Coordinating Center with regulatory documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as needed (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center.
- Submit source documents, research records, and CRFs per protocol specific submission guidelines to the Coordinating Center.
- Submit Serious Adverse Event (SAE) reports to local IRB per local requirements and provide copies to the Coordinating Center, in accordance with DF/HCC requirements.
- Submit SAE reports directly to CTEP and provide copies to the Coordinating Center
- Submit protocol deviations and violations to local IRB per local requirements and provide copies to the Coordinating Center, in accordance with DF/HCC requirements.
    - Deviations should be prospectively approved by the Protocol Chair as per DFCI guidelines.
- Secure and store investigational agents and other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.
- Order their own investigational agents regardless of the supplier (i.e. National Cancer Institute [NCI]).

3.0 DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

The following section will clarify DF/HCC Requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Distribution

The Coordinating Center will distribute the final DFCI IRB approved protocol and any subsequent amended protocols to all Participating Institutions.

3.2 Protocol Revisions and Closures

The Participating Institutions will receive notification of protocol revisions and closures from the Coordinating Center. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within 90 days from receipt of the notification.

- **Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval.

- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the

Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Institution consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Institution.

Participating Institutions are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. Participating institutions must follow the DF/HCC requirement that only attending physicians obtain informed consent and re-consent to interventional trials (i.e. drug and/or device trials).

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

- Approval letter of the Participating Institution's IRB
- Copy of the Informed Consent Form approved by the Participating Institution’s IRB
- Participating IRB’s approval for all amendments

It is the Participating Institution's responsibility to notify its IRB of protocol amendments. Participating Institutions will have 90 days from receipt to provide the Coordinating Center their IRB approval for amendments to a protocol.

3.5 IRB Re-Approval

Verification of IRB re-approval from the Participating Institutions is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received from the Participating Institution on or before the anniversary of the previous approval date.

3.6 Participant Confidentiality and Authorization Statement

In 1996, congress passed the first federal law covering the privacy of health information known as the Health Insurance Portability and Accountability Act (HIPAA). Any information, related to the physical or mental health of an individual is called Protected Health Information (PHI). HIPAA outlines how and under what circumstances PHI can be used or disclosed.

In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an Authorization. This Authorization may or may not be

separate from the informed consent document. The Coordinating Center, with the approval from the DFCI IRB and CTEP, will provide a consent template, which covered entities (Participating Institutions) must use.

The Protocol Chair will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be collected per NCI requirements. These are the primary reasons why DF/HCC has chosen to use Authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

3.6.1 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center must have the participant's full name & social security number "blacked out" and the assigned DF/HCC QACT case number (as described below) and DF/HCC protocol number written in (with the exception of the signed informed consent document). Participant initials may only be included or retained for cross verification of identification

3.7 DF/HCC Multi-Center Protocol Registration Policy

3.7.1 Participant Registration and Randomization

*Eligible participants will be registered onto the trial with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Registration should occur during QACT's no*rmal business hours, Monday through Friday from 8:00 AM to 5:00 PM Eastern Time. Same day treatment registrations and off-hours registrations for a participant at a non-DF/HCC site will only be accepted with prior notice and discussion with the Coordinating Center.

3.7.1.1 Participant Registration at a non-DF/HCC site

Eligibility participants will be entered on study centrally at the Coordinating Center by the Study Coordinator. All sites should call the Study Coordinator to verify dose level availabilities. The required forms mentioned below will be provided to sites by the Coordinating Center during site activation. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be cancelled. The Study Coordinator should be notified of cancellations as soon as possible.

To register a participant at any non-DF/HCC site, the subsequent procedures must be followed. Contact information for the Coordinating Center is located in section 2.2.1.

1. The Participant Site should contact the Coordinating Center to –
   - Notify regarding the potential participant
   - Confirm the methods of sending documents and communication for the registration

- Communicate desired timeline of the registration and start date

2. The Participating Site should then send the following documents to the Coordinating Center –
   - Completed DF/HCC Eligibility Source Worksheet (ESW), which will be provided to each site separately
   - Copy of all protocol required screening test results, as requested on the ESW
   - Copy of the pathology and surgical reports, as requested on the ESW
   - List of all concomitant medications with review by a clinician
   - Copy of the signed informed consent document
   - Copy of the signed HIPAA authorization form (if separate from the informed consent document)
3. After having received all documentation, the Coordinating Center will review the documents to verify eligibility.
4. The Coordinating Center will register the participant with QACT, and subsequently inform the participating site of the successful registration via fax or email, to include
   - Participant case number
   - Applicable dose treatment level
5. **Treatment may not begin without confirmation from the Coordinating Center that the participant has been registered.**
6. After the participant is registered, additional documentation is to be sent to the Coordinating Center if it was not included in the upfront submission. The specifics and timeline of this will be specified by the Coordinating Center within the instructions for the Eligibility Source Worksheet.

NOTE: Registration and randomization with the QACT can only be conducted during the business hours of 8:00 AM and 5:00 PM Eastern Standard Time Monday through Friday. Same day treatment registrations will only be accepted with prior notice and discussion with the DF/HCC Lead Institution.

3.7.1.2 Participant Registration at a DF/HCC site

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. In emergency situations when a participant must begin protocol therapy during off-hours or holidays, call the QACT registration line at 617-632-3761 and follow the instructions for registering participants after hours.

An investigator will confirm eligibility crtieria and a member of the study team will complete the QACT protocol-specific eligibility cehcklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be cancelled. Notify the QACT Registrar of registration cancellations as soon as possible.

To register a participant at a DF/HCC site, the subsequent procedures must be followed. Contact information for the Coordinating Center is located in section 2.2.1.

- Obtain written informed consent from the participant prior to the performance of any protocol specific procedures or assessments
- Complete the QACT protocol-specific eligibility checklist using the eligibility assessment document in the participant's medical record and/or research chart. **To be eligible for registration to the protocol, the participant must meet all inclusion and exclusion criterion as described in the protocol and reflected on the eligibility checklist.**
- Fax the eligibility checklist and all pages of the consent form to the QACT at 617-632-2229.
- The QACT Registrar will (a) review the eligibility checklist, (b) register the participant on the protocol, and (c) randomize the participant when applicable.
- An email confirmation of the registration and/or randomization will be sent to the Overall PI, study coordinator(s) from the Lead Site, treating investigator and registering person immediately following the registration and/or randomization.

3.7.2 Initiation of Therapy

Participants must be registered with the DF/HCC QACT before receiving treatment. Treatment may not be initiated until the Participating Institution receives a faxed or e-mailed copy of the participant's registration confirmation memo from the Coordinating Center. Therapy must be initiated per protocol guidelines. The Coordinating Center and DFCI IRB must be notified of any exceptions to this policy.

3.7.3 Eligibility Exceptions

CTEP specifically prohibits registration of a participant on any NCI Sponsored protocol that does not fully and completely meet all eligibility requirements. The DF/HCC QACT will make no exceptions to the eligibility requirements for a protocol without DFCI IRB approval. The DF/HCC QACT requires each institution to fully comply with this requirement.

3.7.4 Verification of Registration, Dose Levels, and Arm Designation

A registration confirmation memo for participants registered to DF/HCC Multi-Center Protocol will be faxed or emailed to the registering institution within one business day of the registration. Treatment may not be initiated until the site receives a faxed or e-mailed copy of the registration confirmation memo.

3.8 DF/HCC Protocol Case Number

Once eligibility has been established and the participant successfully registered, the participant is assigned a five digit protocol case number. This number is unique to the participant on this trial and must be used for QACT CRF/eCRF completion and correspondence, and correspondence with the Coordinating Center.

3.9 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except

when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the Protocol Chair, who in turn is responsible for reporting to the DFCI IRB.

For reporting purposes, DF/HCC uses the terms "violation", "deviation" and "exception" to describe derivations from a protocol. All Participating Institutions must adhere to these requirements for reporting to the Protocol Chair and will follow their institutional policy for reporting to their local IRB.

3.9.1 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is prospectively approved prior to its implementation.

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.

Protocol Violation: Any protocol deviation that was not prospectively approved by the IRB prior to its initiation or implementation.

3.9.2 Reporting Procedures

Protocol Chair: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations.

All protocol violations must be sent to the Protocol Chair and Coordinating Center in a timely manner.

3.10 Safety Assessments and Toxicity Monitoring

The study teams at all participating institutions are responsible for protecting the safety, rights and well-being of study participants. Recording and reporting of adverse events that occur during the course of a study help ensure the continuing safety of study participants.

All participants receiving investigational agents and/or other protocol mandated treatment will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol. Life-threatening toxicities must be reported immediately to the Protocol Chair via the Coordinating Center. These toxicities must also be reported via the CTEP-AERS system as outlined in the protocol.

Additional safety assessments and toxicity monitoring will be outlined in the protocol.

3.10.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol section 7.

The Coordinating Center will maintain documentation of all Participating Institution Adverse Event reports that are reported to CTEP via CTEP-AERS. The Coordinating Center will also be

responsible for communicating all relevant CTEP SAEs reported via CTEP-AERS to all participant sites.

3.10.2 Guidelines for Processing IND Safety Reports

FDA regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any adverse experience associated with the use of the investigational agent that is both serious and unexpected. The Protocol Chair will review all IND Safety Reports and ensure that all IND Safety Reports are distributed to the Participating Institutions. The Participating Investigators are to review, send a copy to their IRB according to their local IRB's policies and procedures, and file a copy with their regulatory documents.

3.11 Data Management

The DF/HCC QACT develops a set of either paper or electronic case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. The DF/HCC QACT provides a web based training for eCRF users.

3.11.1 Data Forms Review

When data forms arrive at the DF/HCC QACT, they are reviewed for completeness, protocol treatment compliance, adverse events (toxicities) and response. Data submissions are monitored for timeliness and completeness of submission. Participating Institutions are notified of their data submission delinquencies in accordance with the following:

<u>Incomplete or Questionable Data</u>
If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC QACT Data Analyst or study monitor. Responses to all queries should be completed and submitted within 14 calendar days. Responses may be returned on the written query or on an amended paper case report form, or in the case of electronic queries, within the electronic data capture (eDC) system. In the case of a written query for data submitted on a paper case report form, the query must be attached to the specific data being re-submitted in response.

<u>Missing Forms</u>
If study forms are not submitted on schedule, the Participating Institution will receive a Missing Form Report from the Coordinating Center noting the missing forms. These reports are compiled by the DF/HCC QACT and distributed a minimum of four times a year.

4.0 Requisitioning Investigational Drug

The ordering of investigational agent is specified in protocol section 8.

Participating Institutions should order their own agent.

For commercially available agents (bevacizumab), participant institutions should check with the Research Pharmacy to ensure that the agent is in stock. If the agent is not stocked, ensure that the agent can be ordered once the protocol is approved by the local IRB.

For investigational agents (MLN0128), participating institutions should ensure that the pharmacy will be able to receive and store the agent according to state and federal requirements. The local IRB

should be kept informed of who will supply the agent (NCI) so that any regulatory responsibilities can be met in a timely fashion.

4.1 CTEP Trial Routine Drug Requisitions

Investigational drugs for use in Cancer Therapy Evaluation Program's (CTEP) approved protocols are obtained through the Pharmaceutical Management Branch at CTEP in accordance with the guidelines provided in the NCI's Investigator Handbook. Participating Institutions should order drugs directly from Division of Cancer Treatment and Diagnosis (DCTD) by completing a Clinical Drug Request (CDR) Form NIH-986.

4.1.1 Guidelines for Clinical Drug Request Submission

NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agents be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of Form FDA 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application (https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://eapps-ctep.nci.nih.gov/iam/) and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

5.0 MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires verification of protocol compliance and data accuracy. NCI/CTEP, the DFCI Coordinating Center, and DF/HCC QACT provide quality control oversight for the protocol.

5.1 Monitoring by NCI/CTEP

The NCI will utilize a variety quality control procedures:

- Built-in edit checks within the Electronic Data Capture System
- Cross check of data between various electronic reporting systems
- Site performance evaluations
- Special Response reviews to verify outcome data
- Committees for central review of major elements that impact on the outcome of clinical trials, e.g., pathology, radiotherapy, surgery, and administration of investigational agents; and
- Educational functions which address data collection, data management, and overall data quality

5.2 Monitoring by DFCI Coordinating Center

The Participating Institutions are to submit participant source documents to the Coordinating Center for virtual monitoring upon request. Participating Institution may also be subject to on-site monitoring conducted by the Coordinating Center. In addition, participating DF/HCC sites, including the lead institution, will be subject to monitoring as well.

The Coordinating Center will implement ongoing monitoring activities to ensure that sites 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, 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.

Additionally, sites will be required to participate in Coordinating Center initiated teleconferences. These will be planned for bi-weekly during Stage 1 of the protocol and monthly during Stage 2, although they may be more/less frequent at the discretion of the Protocol Chair. During the teleconferences, sites will be expected to convey the following information:

- Updates on participants taking agent: holds, dose reductions, significant events, how the participant is doing, whether or not underwent re-consenting
- Protocol status – which version is being used, and the status of any amendments
- Any reportable adverse events or deviations/violations that have yet to be submitted
- Review of prospective patients

If sites are not able to have a representative participant, they should email this information to the Coordinating Center.

Virtual Monitoring will be the primary mode of monitoring, although on-site monitoring may occur if felt to be necessary by the Coordinating Center and Protocol Chair. Upon request, sites will be required to forward de-identified copies of participants' medical record and source documents to the Coordinating Center to aid in source data verification.

- Virtual monitoring of participant eligibility, human subject's protection via the initial informed consent document and process, and screening evaluation completion will occur, if feasible, within 2 weeks of the first participant registration.
- Interim monitoring visits will occur on the following schedule:
    - Once a site has registered a participant, up until all participants have discontinued taking study agent (may be in follow-up), interim monitoring visits will occur approximately every 6 months, with the primary mode being virtual visits. This frequency may be adjusted depending on accrual, site compliance and Protocol Chair discretion. The first interim monitoring visit will occur approximately two months after the registration of the site's first participant.
    - Once a site is closed to accrual and all participants have discontinued study agent, interim monitoring visits will occur approximately annually until study completion.
- Monitoring visits will focus on reviewing some or all of the following:
    - Adverse events and altered results
    - Response assessment including measurements and clinical assessments
    - Study drug administration and accountability
    - Concomitant medications

- Consent and re-consenting
- Presence of key documents: original consent, eligibility and screening source information, registration confirmation, off treatment and off study forms, collection and transfer of samples
- Reason off treatment and reason off study
- Timeliness of data completion
- Completion of study procedures per protocol
- Agreement between recorded results and source documentation
- Analysis of data for any events that meet criteria for reportable adverse events, dose holds, dose reductions, or discontinuation of treatment
- Regulatory binder: accessibility, organization, random sampling for relevant documents and agreement with the trial master file
- Collection of research samples per protocol and appropriately entered onto CRFs, as this will be the primary mode of tracking samples across sites

The Coordinating Center will be available to all sites' study team members for resolving questions and concerns and facilitating compliance.

All data submitted to the DF/HCC QACT will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. The Coordinating Center and if applicable, QACT Data Analysts assigned to the protocol, will perform the ongoing protocol data compliance monitoring.

5.3 Evaluation of Participating Institution Performance

5.3.1 Monitoring Reports

The Protocol Chair will review all monitoring reports for on-site and virtual monitoring of sites to ensure protocol compliance and ability to fulfill responsibilities of participating in the study. The Protocol Chair may increase the monitoring activities at a particular site that is unable to comply with the protocol, DF/HCC or CTEP requirements or federal and local regulations. Participating sites may also be subject to an audit as determined by the Protocol Chair.

6.0 AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance. Its main focus is to measure whether standards and procedures were followed. Auditing is the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and whether data was generated, recorded and analyzed, and accurately reported per the protocol, Standard Operating Procedures (SOPs), and the Code of Federal Regulations (CFR).

6.1 NCI Sponsored Trials

The Clinical Trials Monitoring Branch (CTMB) of the National Cancer Institute may arrange on-site auditing for this study. Therefore, in some cases the Protocol Chair funded audits may not occur on-site.

Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source

documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

Participating Institutions that are not a part of a major cancer center may not undergo the routine NCI/Theradex audits. In those cases, the Participating Institution may be subject to on-site auditing conducted by the Coordinating Center.

6.2 Participating Institution

It is the Participating Institution's responsibility to notify the Coordinating Center of all scheduled audit dates (internal or NCI) and re-audit dates (if applicable), which involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

6.3 Protocol Chair and Coordinating Center

The Protocol Chair will review all final audit reports and corrective action plans if applicable. The Coordinating Center, must forward these reports to the DF/HCC QACT per DF/HCC policy for review by the DF/HCC Audit Committee. Based upon the audit assessments the DF/HCC Audit Committee could accept or conditionally accept the audit rating and final report. Conditional approval could require the Protocol Chair to implement recommendations or require further follow-up.

6.4 Sub-Standard Performance

The Protocol Chair, DFCI IRB and the NCI for CTEP trials, is charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

6.4.1 Corrective Actions

Participating Institutions that fail to meet the performance goals of accrual, submission of timely accurate data, adherence to protocol requirements, and compliance with state and federal regulations, will be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the Protocol Chair for revocation of participation.

APPENDIX E DOSE LEVEL 1, 2 & 3 PILL DIARY FOR MLN0128

Completed by study personnel: Participant ID #_______ Participant Initials: _______ Cycle #: ______ Month & Year: _________
MLN0128 Daily Dose________mg/day

To be completed by Participant or Guardian:

Number of Pill Bottles Received:______ with 1 mg capsules; _______with 3 mg capsules; :______ with 5 mg capsules

DAY:	**DAY:**	**DAY:**	**DAY:**	**DAY:**	**DAY:**	**DAY:**
Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________
Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ No. of 5 mg pills _____ Participant Initials _________

DAY:	DAY:	DAY:	DAY:	DAY:	DAY:	DAY:
Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________	Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________	Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________	Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________	Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________	Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________	Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________
Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________	Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________	Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________	Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________	Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________	Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________	Date: Time: No. of 1 mg pills ______ No. of 3 mg pills ______ No. of 5 mg pills ______ Participant Initials __________

Participant/Guardian Signature: ___________________________________ ***Date:*** _________________

To be completed by study personnel: Bottles Returned: ___with 1 mg capsules; ___with 3 mg capsules; ___with 5 mg capsules

Pills Returned: ___ 1 mg; ___3 mg; ___5 mg

Compare w/ drug diary entries made by participant or guardian. If discrepancy (in the # of bottles or the # of pills returned), please reconcile:

Study Personnel Signature:__________________________________ **Date:** __________________

MLN0128 Dosing Instruction Sheet
to Be Given to Study Participants with Participant Pill Diary

MLN0128 Instructions – When and How:

- MLN0128 is an oral drug.
- Take MLN0128 every morning after a light meal at approximately the same time each day.
- Take the capsule(s) with a glass of water and swallow your dose whole. Do not chew them or suck on the capsules. Capsules should not be opened. If you chew, suck or the capsule(s) open in your mouth, you should drink a large glass of water (about 8 ounces).
- Please try to drink at least 18-24 ounces of liquids a day to stay well-hydrated.
- If you vomit your dose, do not take another dose; you will take your next dose on the next day as usual. You should write down in your pill diary that you vomited the pills.
- If you forget to take your dose at your normal dosing time, you may still take the dose within 12 hours of the regular dosing time with a meal (you should not take 2 consecutive daily doses within less than 12 hours of each other).
- If you miss your dose more than 12 hours after your regular dosing time you should write down in your pill diary that you missed the dose.

Additional Instructions:

- You must avoid eating or drinking any foods with Seville oranges (like marmalade), grapefruit or foods or drink with grapefruit, and any exotic fruits during the entire treatment period.
- Keep your study drug in the original container.
- Do not throw away empty pill bottles.
- Bring this diary, all pill bottles, and any unused pills to all scheduled clinic visits.
- Contact your study doctor or nurse if you are having any new or worsening side effects.

Please note that on days that you are coming to clinic or having blood tests, you may need to take your pills in clinic visit because of required study tests.

APPENDIX F DOSE LEVEL 1A & -1 PILL DIARY FOR MLN0128

Completed by study personnel: Participant ID #_______ Participant Initials: _______ Cycle #: ______ Month & Year: _________

MLN0128 Dose________mg/day 5 of 7 days

To be completed by Participant or Guardian:

Number of Pill Bottles Received:______ with 1 mg capsules; _______with 3 mg capsules

DAY:	**DAY:**	**DAY:**	**DAY:**	**DAY:**	**DAY:**	**DAY:**
Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: No pills taken today.	Date: No pills taken today.
Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: No pills taken today.	Date: No pills taken today.

DAY:	DAY:	DAY:	DAY:	DAY:	DAY:	DAY:
Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: No pills taken today.	Date: No pills taken today.
Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: Time: No. of 1 mg pills _____ No. of 3 mg pills _____ Participant Initials _________	Date: No pills taken today.	Date: No pills taken today.

Participant/Guardian Signature: ________________________________ **Date:** _______________

To be completed by study personnel: Bottles Returned: __with 1 mg capsules; __with 3 mg capsules; Pills Returned: ___ 3 mg; ___4 mg
Compare w/ drug diary entries made by participant or guardian. If discrepancy (in the # of bottles or the # of pills returned), please reconcile:

Study Personnel Signature:_______________________________ **Date:** ________________

MLN0128 Dosing Instruction Sheet

to Be Given to Study Participants with Participant Pill Diary

MLN0128 Instructions – When and How:

- MLN0128 is an oral drug.
- Take MLN0128 every morning after a light meal at approximately the same time each day.
- Take the capsule(s) with a glass of water and swallow your dose whole. Do not chew them or suck on the capsules. Capsules should not be opened. If you chew, suck or the capsule(s) open in your mouth, you should drink a large glass of water (about 8 ounces).
- Please try to drink at least 18-24 ounces of liquids a day to stay well-hydrated.
- If you vomit your dose, do not take another dose; you will take your next dose on the next day as usual. You should write down in your pill diary that you vomited the pills.
- If you forget to take your dose at your normal dosing time, you may still take the dose within 12 hours of the regular dosing time with a meal (you should not take 2 consecutive daily doses within less than 12 hours of each other).
- If you miss your dose more than 12 hours after your regular dosing time you should write down in your pill diary that you missed the dose. Do not take your missed dose on your rest days, the shaded days of your diary.

Additional Instructions:

- You must avoid eating or drinking any foods with Seville oranges (like marmalade), grapefruit or foods or drink with grapefruit, and any exotic fruits during the entire treatment period.
- Keep your study drug in the original container.
- Do not throw away empty pill bottles.
- Bring this diary, all pill bottles, and any unused pills to all scheduled clinic visits.
- Contact your study doctor or nurse if you are having any new or worsening side effects.

Please note that on days that you are coming to clinic or having blood tests, you may need to take your pills in clinic visit because of required study tests.

APPENDIX G BIOASSAY PROCEDURES

Proteomics Program Standard Operating Procedure for Tissue Core Collection- Needle Biopsy – Cryopreservation in OCT

Principle:
Core needle biopsies are used to sample tissue from a specific, defined location. These biopsies may consist of normal, pre-malignant and malignant tissue due to the multi-level tissue sample that is obtained. This type of sample is ideal for studying the micro-tumor environment. Rapid freezing of the sample is required to prevent degradation of the proteins or RNA. Optimal Cutting Temperature (OCT) compound is an alcohol polymer that is liquid at room temperature and a solid at –20C. This polymer is used to cryo-protect the tissue and provide a medium for cryo-sectioning. The goal for time of tissue acquisition to freezing is <10 minutes.

Materials:
Cryomolds (Sakura Finetek Cat. # 4728)
OCT (Sakura Finetek Cat. # 4583)
Dry ice
Ultra cold freezer (-700 to -800C)
Needle: 16 or 18 gauge
Permanent marker
Sterile forceps
Sterile Glass slides
Aluminum foil or 50ml Falcon tubes

Procedure:
1. Prepare all supplies prior to the biopsy procedure to avoid delay once the specimen has been obtained.
2. Label the handle and the front surface of the cryomold with the sample or patient's identifying information.
3. Perform core needle biopsy.
4. Pick the core from the biopsy needle onto a sterile glass slide.
5. Fill cryomold about 1/3 full with OCT. Place the cryomold in dry ice to partially freeze the OCT. The OCT should be jelly-like, not completely frozen.
6. Carefully lift the core biopsy by both ends with sterile forceps. **Do not stretch the biopsy or it will break.**
7. Lay the biopsy as straight as possible in the OCT. Once the sample touches the OCT, you cannot reposition it or the sample will break apart.
8. Quickly add OCT on top of the biopsy, completely covering the sample.
9. Ensure the sample is level and freeze immediately in dry ice.
10. Store wrapped in aluminum foil or in a 50ml Falcon tube at –70C.

Note:
Do not lay the biopsy on frozen OCT and cover it with liquid OCT. The OCT will not fuse and will split into two sections when cutting the frozen tissue sections.

Frozen Section Slides
1. Frozen sections for proteomic analysis should be cut at 5-8um on plain, uncoated glass microscope slides.
2. The tissue section should be placed as close as possible to the center of the slide. Do not place the frozen section at the end of the slide.
3. Two tissue sections from the same biopsy may be placed on the same glass slide if space permits.
4. Do not allow the tissue section to air on the slide. Freeze immediately on dry ice or at –80C.

REVERSE PHASE PROTEIN MICROARRAY

Rationale

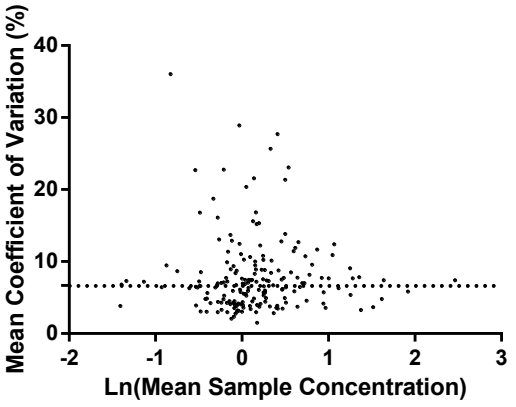
Molecular therapeutics is a relatively novel approach that targets abnormalities in signaling pathways that play critical roles in tumor development and progression. Kinases and phosphatases control the reversible process of phosphorylation and are dysregulated in many diseases including cancer. Protein and lipid phosphorylation regulates cell survival signaling. Targeting kinases and phosphatases are paramount for improving therapeutic intervention. In this regard, it is critical to define qualified cellular targets for cancer diagnoses and prognoses, as well as accurately predict and monitor responsiveness to therapies.

The reverse phase protein microarray (RPPA) is a recently developed quantitative assay that analyzes nanoliter amounts of sample for potentially hundreds of proteins. This antibody-based assay determines levels of protein expression, as well as protein modifications such as phosphorylation, cleavage, and fatty acid modification. RPPAs allow concordant interrogation of multiple signaling molecules and their functional status. RPPAs have been utilized to profile and validate signaling networks in human cancer cell lines and tumor tissue[1-3].

In essence, the RPPA study has major strengths in identification and validation of cellular targets, characterization of signaling pathways and networks, as well as determination of on and off target activity of novel drugs. The integrated information will display the potential therapeutic targets or biomarkers to accurately predict or rapidly define intracellular signaling networks and functional outcomes affected by therapeutics. Thus, the RPPA approach provides a method to assess multiple markers in a global manner. Linking robust pathway mapping approaches to molecular therapeutics should provide an expanding repertoire of validated biomarkers and targeted therapeutics for clinical evaluation.

Availability of key reagents required for execution of the high throughput RPPA project

We have extensively validated antibodies suitable for RPPA (please refer to the attached list of RPPA-validated antibodies). Only antibodies with a single or dominant band on western blotting are further assessed by direct comparison to RPPA using cell lines with differential protein expression or modulated with ligands/inhibitors or siRNA for phospo- or structural proteins, respectively. Only antibodies with a Pearson correlation coefficient greater than 0.7 between RPPA and western blotting as well as wide dynamic range will be used. Further, these antibodies are assessed for specificity and quantification using phospho- and non-phosphopeptides arrayed on nitrocellulose-coated slides. Interslide reproducibility is high with average coefficient of variation for individual antibodies across multiple cell lines ranging from 1.5 - 36.1%, median 6.7% (figure 1).



We have full access to a Tecan robotic liquid handling system for serial dilution of cell lysates and sample transfer; a G3 GeneTac arrayer and an Aushon 2470 arrayer for printing of up to 24 (G3) or 100 slides (2470) per run with several automated runs feasible in a day; a Biogenix i600TM and two DAKO Universal Staining systems that probes each slide with a different antibody. Each autostainer is capable of staining approximately 60 slides per day under conditions that are specific for each individual antibody.

Approach

For tumor tissue, we will extract proteins from 8-10 mg of snap frozen tissue by homogenizer or ceramic beads (See appendix D for description of biopsy handling and below for lysis procedure). We will also include one ovarian and one endometrial cancer cell line (untreated and treated with multiple concentrations of MLN0128) that will be handled identically. Protein concentration will be determined and adjusted to 1ug/ul. Protein will be denatured by SDS.

Tissue lysates will be serial diluted to define the linear range of each antigen-antibody reaction. Lysates will be printed on nitrocellulose-coated slides and probed with validated antibodies that recognize signaling molecules or their activated forms. Signals will be captured by tyramide dye deposition (CSA System, DAKO).

Data will be collected and analyzed using quantification software specifically developed for this approach (http://www.vigenetech.com); this software has multiple features that have been developed by the company in collaboration with the MDACC RPPA Core Laboratory including automated spot identification, background correction, controlling for location, serial dilution-signal intensity curve construction and concentration determination. The values derived from the slope and intercept will be expressed relative to standard control cell lysates or control peptides on the array.

Relative protein levels for each sample will be determined by interpolation of each dilution curve from the "standard curve" (supercurve) of the slide (antibody). Each dilution curve will be fitted with a logistic model ("Supercurve Fitting" developed by the Department of Bioinformatics and Computational Biology in MD Anderson Cancer Center, "http://bioinformatics.mdanderson.org/OOMPA"). This fits a single curve using all the samples (i.e., dilution series) on a slide with the signal intensity as the response variable and the dilution steps are independent variable. The fitted curve is plotted with the signal intensities – both observed and fitted - on the y-axis and the log2-concentration of proteins on the x-axis for diagnostic purposes. The protein concentrations of each set of slides will then be normalized by median polish, which is corrected across samples by the linear expression values using the median expression levels of all antibody experiments to calculate a loading correction factor for each sample. These values (given as log2 values) will be reported in the "SprCrvRaw (log2)" data set. All of the data points will be normalized for protein loading and transformed to linear values as indicated in the "Normalized Linear" data set. For antibodies with replicated slides, we will choose the one with the highest QC Scores. The QC Scores and antibody correlations will be included in the data set for reference.

1. Yang JY, Yoshihara K, Tanaka K, et al. Predicting time to ovarian carcinoma recurrence using protein markers. J Clin Invest 2013;123:5410.
2. Cardnell RJ, Feng Y, Diao L, et al. Proteomic Markers of DNA Repair and PI3K Pathway Activation Predict Response to the PARP Inhibitor BMN 673 in Small Cell Lung Cancer. Clin Cancer Res 2013;19:6322-8.
3. Sohn J, Do KA, Liu S, et al. Functional proteomics characterization of residual triple-negative breast cancer after standard neoadjuvant chemotherapy. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2013;24:2522-6.

Core Standard Antibody List (as of 11/2013)

#	Ab Name	Gene Name	Company	Catalog #	Internal Ab ID	Species	Validation Status*
1	14_3_3_beta	YWHAB	Santa Cruz	sc-628	882	Rabbit	Validated
2	14_3_3_epsilon	YWHAE	Santa Cruz	sc-23957	913	Mouse	Use with Caution
3	14_3_3_zeta	YWHAZ	Santa Cruz	sc-1019	883	Rabbit	Validated
4	4E BP1	EIF4EBP1	CST	9452	2	Rabbit	Validated
5	4E BP1_pS65	EIF4EBP1	CST	9456	3	Rabbit	Validated
6	4E BP1_pT37/T46	EIF4EBP1	CST	9459	6	Rabbit	Validated
7	53BP1	TP53BP1	CST	4937	985	Rabbit	Validated
8	A Raf	ARAF	CST	4432	1217	Rabbit	Validated
9	ACC_pS79	ACACA ACACB	CST	3661	13	Rabbit	Validated
10	ACC1	ACACA	Epitomics	1768-1	14	Rabbit	Under Evaluation
11	ACVRL1	ACVRL1	Abcam	ab108207	1086	Rabbit	Use with Caution
12	ADAR1	ADAR1	Abcam	ab88574	1198	Mouse	Validated
13	Akt	AKT1 AKT2 AKT3	CST	4691	1084	Rabbit	Validated
14	Akt_pS473	AKT1 AKT2 AKT3	CST	9271	230	Rabbit	Validated
15	Akt_pT308	AKT1 AKT2 AKT3	CST	2965	1154	Rabbit	Validated
16	AMPK_alpha	PRKAA1	CST	2532	39	Rabbit	Use with Caution
17	AMPK_pT172	PRKAA1	CST	2535	40	Rabbit	Validated
18	Annexin I	ANXA1	BD Biosciences	610066	1208	Mouse	Validated
19	Annexin VII	ANXA7	BD Biosciences	610668	1142	Mouse	Validated
20	AR	AR	Epitomics	1852-1	756	Rabbit	Validated
21	ARHI	DIRAS3	MDACC Laboratory	Bast Lab	1273	Mouse	Use with Caution
22	ATM	ATM	CST	2873	1363	Rabbit	Validated
23	ATM_pS1981	ATM	CST	5883	1364	Rabbit	Validated
24	ATP5H	ATP5H	Abcam	ab110275	1252	Mouse	Use with Caution
25	B Raf	BRAF	Santa Cruz	sc-5284	96	Mouse	Use with Caution
26	B Raf_pS445	BRAF	CST	2696	94	Rabbit	Validated
27	Bad_pS112	BAD	CST	9291	63	Rabbit	Validated
28	Bak	BAK1	Epitomics	1542-1	71	Rabbit	Use with Caution
29	BAP1	BAP1	Santa Cruz	sc-28383	1207	Mouse	Validated
30	Bax	BAX	CST	2772	73	Rabbit	Validated
31	Bcl 2	BCL2	Dako	M0887	80	Mouse	Validated
32	Bcl xL	BCL2L1	CST	2762	85	Rabbit	Validated
33	Beclin	BECN1	Santa Cruz	sc-10086	87	Goat	Use with Caution
34	Beta_catenin	CTNNB1	CST	9562	75	Rabbit	Validated
35	Beta_catenin_pT41/S45	CTNNB1	CST	9565	1170	Rabbit	Validated
36	Bid	BID	Abcam	ab32060	88	Rabbit	Use with Caution
37	Bim	BCL2L11	Abcam	ab32158	90	Rabbit	Validated
38	BRcA2	BRCA2	CST	9012	761	Rabbit	Use with Caution
39	c Jun_pS73	JUN	CST	9164	155	Rabbit	Validated
40	c Kit	KIT	Abcam	ab32363	157	Rabbit	Validated
41	c Met_pY1235	MET	CST	3129	727	Rabbit	Validated
42	c Myc	MYC	Santa Cruz	sc-764	1143	Rabbit	Use with Caution
43	c Raf	RAF1	Millipore	04-739	1201	Rabbit	Validated
44	c Raf_pS338	RAF1	CST	9427	179	Rabbit	Validated
45	Caspase 7_cleavedD198	CASP7	CST	9491	109	Rabbit	Use with Caution
46	Caveolin 1	CAV1	CST	3238	114	Rabbit	Validated
47	CD29	CD29	BD Biosciences	610467	1206	Mouse	Validated
48	CD31	PECAM1	Dako	M0823	127	Mouse	Validated
49	CD49b	ITGA2	BD Biosciences	611016	937	Mouse	Validated
50	CDK1	CDC2	CST	9112	1007	Rabbit	Validated
51	CDKN2A/p16INK4a	CDKN2A	Abcam	ab81278	1231	Rabbit	Validated
52	Chk1	CHEK1	CST	2360	1203	Mouse	Use with Caution
53	Chk1_pS345	CHEK1	CST	2348	903	Rabbit	Use with Caution
54	Chk2	CHEK2	CST	3440	146	Mouse	Validated
55	Chk2_pT68	CHEK2	CST	2197	147	Rabbit	Use with Caution
56	cIAP	BIRC2	Millipore	07-759	930	Rabbit	Use with Caution
57	Claudin 7	CLDN7	Novus	NB100-91714	852	Rabbit	Validated
58	Collagen VI	COL6A1	Santa Cruz	sc-20649	171	Rabbit	Validated

59	Complex II_Subunit 30	MED30	Invitrogen	459230	1069	Mouse	Validated
60	Cox 2	CMC2	CST	4842	1218	Rabbit	Use with Caution
61	Cox IV	PTGS3	Abcam	ab14744	1253	Mouse	Use with Caution
62	Cyclin B1	CCNB1	Epitomics	1495-1	192	Rabbit	Validated
63	Cyclin D1	CCND1	Santa Cruz	sc-718	194	Rabbit	Validated
64	Cyclin E1	CCNE1	Santa Cruz	sc-247	201	Mouse	Validated
65	Cyclophilin F	PPIF	Abcam	ab110324	1257	Mouse	Validated
66	DJ 1	PARK7	Abcam	ab76008	891	Rabbit	Validated
67	Dvl 3	DVL3	CST	3218	940	Rabbit	Validated
68	E cadherin	CDH1	CST	3195	1099	Rabbit	Validated
69	E2F1	E2F1	Santa Cruz	sc-251	1261	Mouse	Validated
70	eEF2	EEF2	CST	2332	1060	Rabbit	Use with Caution
71	eEF2K	EEF2K	CST	3692	1061	Rabbit	Validated
72	EGFR	EGFR	CST	2232	1120	Rabbit	Validated
73	EGFR_pY1068	EGFR	CST	2234	217	Rabbit	Use with Caution; also sees pHer2
74	EGFR_pY1173	EGFR	Abcam	ab32578	221	Rabbit	Validated
75	eIF4E	EIF4E	CST	9742	722	Rabbit	Validated
76	eIF4G	EIF4G1	CST	2498	1124	Rabbit	Use with Caution
77	ER alpha	ESR1	Lab Vision	RM-9101-S	238	Rabbit	Validated
78	ER alpha_pS118	ESR1	Epitomics	1091-1	241	Rabbit	Validated
79	ETS 1	ETS1	Bethyl	A303-501A	1200	Rabbit	Validated
80	FAK	FAK	CST	3285	1228	Rabbit	Under Evaluation
81	FAK_pT397	PTK2	CST	3283	1227	Rabbit	Validated
82	FASN	FASN	Cell Signaling	3180	1156	Rabbit	Validated
83	Fibronectin	FN1	Epitomics	1574-1	262	Rabbit	Validated
84	Fox03a	FOXO3	CST	2497	1122	Rabbit	Use with Caution
85	FoxM1	FOXM1	CST	5436	1123	Rabbit	Validated
86	FoxO3a_pS318/S321	FOXO3	CST	9465	270	Rabbit	Use with Caution
87	G6PD	G6PD	Santa Cruz	sc-373887	1155	Mouse	Validated
88	Gab2	GAB2	CST	3239	943	Rabbit	Validated
89	GAPDH	GAPDH	Ambion	AM4300	274	Mouse	Use with Caution
90	GATA3	GATA3	BD Biosciences	558686	764	Mouse	Validated
91	GCN5L2	KAT2A	CST	3305	1263	Rabbit	Validated
92	GPBB	PYGM	Novus Biologicals	NBP1-32799	1248	Rabbit	Validated
93	GSK3_alpha/beta	GSK3A GSK3B	Santa Cruz	sc-7291	284	Mouse	Validated
94	GSK3_alpha/beta_pS21/S9	GSK3A GSK3B	CST	9331	285	Rabbit	Validated
95	GSK3_pS9	GSK3A GSK3B	CST	9336	1082	Rabbit	Validated
96	GYS	GYS	CST	3886	1035	Rabbit	Validated
97	GYS_pS641	GYS	CST	3891	1036	Rabbit	Validated
98	HER2	ERBB2	Lab Vision	MS-325-P1	1038	Mouse	Validated
99	HER2_pY1248	ERBB2	R&D Systems	AF1768	1075	Rabbit	Use with Caution; likely sees pEGFR
100	HER3	ERBB3	Santa Cruz	sc-285	911	Rabbit	Validated
101	HER3_pY1289	ERBB3	CST	4791	728	Rabbit	Use with Caution
102	Heregulin	NRG1	CST	2573	890	Rabbit	Validated
103	Histone H3	HIST3H3	Abcam	ab1791	1250	Rabbit	Validated
104	IGF1R	IGF1R	CST	3018	1220	Rabbit	Validated
105	IGFBP2	IGFBP2	CST	3922	335	Rabbit	Validated
106	INPP4B	INPP4B	CST	4039	1065	Rabbit	Validated
107	IRS1	IRS1	Millipore	06-248	802	Rabbit	Validated
108	JNK_pT183/Y185	MAPK8	CST	4668	888	Rabbit	Validated
109	JNK2	MAPK9	CST	4672	380	Rabbit	Use with Caution
110	Lck	LCK	CST	2752	397	Rabbit	Validated
111	MAPK_pT202/Y204	MAPK1 MAPK3	CST	4377	405	Rabbit	Validated
112	Mcl 1	MCL1	CST	5453	1222	Rabbit	Validated
113	MDM2_pS166	MDM2	CST	3521	1164	Rabbit	Validated
114	MEK1	MAP2K1	Epitomics	1235-1	417	Rabbit	Validated
115	MEK1_pS217/S221	MAP2K1	CST	9154	1076	Rabbit	Validated
116	MEK2	MAP2K2	CST	9125	1243	Rabbit	Validated

117	MIG 6	ERRFI1	Sigma Aldrich	WH0054206M1	1062	Mouse	Validated
118	MSH2	MSH2	CST	2850	905	Mouse	Validated
119	MSH6	MSH6	Novus Biologicals	22030002	1063	Rabbit	Use with Caution
120	mTOR	FRAP1	CST	2983	444	Rabbit	Validated
121	mTOR_pS2448	FRAP1	CST	2971	446	Rabbit	Use with Caution
122	MYH11	MYH11	Novus Biologicals	21370002	1139	Rabbit	Validated
123	Myosin Iia_pS1943	MYH9	CST	5026	1160	Rabbit	Validated
124	N cadherin	CDH2	CST	4061	452	Rabbit	Validated
125	N Ras	NRAS	Santa Cruz	sc-31	1136	Mouse	Validated
126	Napsin	NAPSA	Abcam	ab129189	1274	Rabbit	Use with Caution
127	NDRG1_pT346	NDRG1	CST	3217	1126	Rabbit	Validated
128	NF2	NF2	Novus Biologicals	22710002	1046	Rabbit	Use with Caution
129	NF-kBp65_pS536	NFKB1	CST	3033	457	Rabbit	Use with Caution
130	Notch1	NOTCH1	CST	3268	1064	Rabbit	Validated
131	p21	CDKN1A	Santa Cruz	sc-397	470	Rabbit	Validated
132	p27	CDKN1B	Abcam	ab32034	897	Rabbit	Validated
133	p27_pT157	CDKN1B	R&D	AF1555	842	Rabbit	Use with Caution
134	p27_pT198	CDKN1B	Abcam	ab64949	878	Rabbit	Validated
135	p38 alpha MAPK	MAPK1	CST	9228	1175	Mouse	Validated
136	p38 MAPK	MAPK14	CST	9212	478	Rabbit	Validated
137	p38_pT180/Y182	MAPK14	CST	9211	479	Rabbit	Validated
138	p53	TP53	CST	9282	481	Rabbit	Use with Caution
139	p70S6K	RPS6KB1	Epitomics	1494-1	493	Rabbit	Validated
140	p70S6K_pT389	RPS6KB1	CST	9205	494	Rabbit	Validated
141	p90RSK	RPS6KA1	CST	9347	759	Rabbit	Use with Caution
142	PAI 1	PAI1	BD Biosciences	612024	499	Mouse	Validated
143	Paxillin	PXN	Epitomics	1500-1	505	Rabbit	Use with Caution
144	PCNA	PCNA	Abcam	ab29	511	Mouse	Use with Caution
145	PDCD 1L1	PDCD1	Santa Cruz	sc-19090	1234	Goat	Use with Caution
146	PDCD4	PDCD4	Rockland	600-401-965	816	Rabbit	Use with Caution
147	PDGFR_beta	PDGFR	CST	3169	1225	Rabbit	Validated
148	PDK1	PDK1	CST	3062	515	Rabbit	Validated
149	PDK1_pS241	PDK1	CST	3061	516	Rabbit	Validated
150	PEA15	PEA15	CST	2780	1017	Rabbit	Validated
151	PEA15_pS116	PEA15	Invitrogen	44-836G	1018	Rabbit	Validated
152	PI3K_p110_alpha	PIK3CA	CST	4255	808	Rabbit	Use with Caution
153	PI3K_p85	PIK3R1	Millipore	06-195	523	Rabbit	Validated
154	PKC_alpha	PRKCA	Millipore	05-154	529	Mouse	Validated
155	PKC_alpha_pS657	PRKCA	Millipore	06-822	530	Rabbit	Use with Caution
156	PKC_delta_pS664	PRKCD	Millipore	07-875	932	Rabbit	Validated
157	PKCpan BetaII_pS660	PKC	CST	9371	1137	Rabbit	Validated
158	PMS2	PMS2	Novus Biologicals	22510002	1246	Rabbit	Validated
159	PR	PGR	Abcam	ab32085	549	Rabbit	Validated
160	PRAS40_pT246	AKT1S1	Biosource	441100G	739	Rabbit	Validated
161	PREX1	PREX1	Abcam	ab102739	1204	Rabbit	Validated
162	PTEN	PTEN	CST	9552	566	Rabbit	Validated
163	Rab11	RAB11A RAB11B	CST	3539	1083	Rabbit	Under Evaluation
164	Rab25	RAB25	CST	4314	1150	Rabbit	Validated
165	Rad50	RAD50	Millipore	05-525	987	Mouse	Validated
166	Rad51	RAD51	CST	8875	1262	Rabbit	Validated
167	Raptor	RPTOR	CST	2280	1128	Rabbit	Validated
168	Rb_pS807/811	RB1	CST	9308	557	Rabbit	Validated
169	RBM15	RBM15	Novus Biologicals	21390002	1138	Rabbit	Validated
170	Rictor	RICTOR	CST	2114	1129	Rabbit	Use with Caution
171	Rictor_pT1135	RICTOR	CST	3806	1130	Rabbit	Validated
172	S6_pS235/236	RPS6	CST	2211	600	Rabbit	Validated
173	S6_pS240/244	RPS6	CST	2215	601	Rabbit	Validated
174	SCD1	SCD1	Santa Cruz	sc-58420	1127	Mouse	Validated
175	SF2	SFRS1	Invitrogen	32-4500	1131	Mouse	Validated
176	SHC_pY317	SHC1	CST	2431	1031	Rabbit	Validated
177	Smad1	SMAD1	Epitomics	1649-1	922	Rabbit	Validated
178	Smad3	SMAD3	Abcam	ab40854	796	Rabbit	Validated
179	Smad4	SMAD4	Santa Cruz	sc-7966	920	Mouse	Validated

180	Src	SRC	Millipore	05-184	621	Mouse	Validated
181	Src_pY416	SRC	CST	2101	623	Rabbit	Use with Caution
182	Src_pY527	SRC	CST	2105	626	Rabbit	Validated
183	STAT3_pY705	STAT3	CST	9131	637	Rabbit	Validated
184	STAT5_alpha	STAT5A	Abcam	ab32043	638	Rabbit	Validated
185	Stathmin	STMN1	Abcam	ab52630	718	Rabbit	Validated
186	Syk	SYK	Santa Cruz	sc-1240	1033	Mouse	Validated
187	TAZ	WWTR1	CST	2149	777	Rabbit	Validated
188	TIGAR	C12ORF5	Abcam	ab137573	1107	Rabbit	Validated
189	Transglutaminase	TGM2	Lab Vision	MS-224-P1	908	Mouse	Validated
190	TRFC	TRFC	Novus Biologicals	22500002	1140	Rabbit	Validated
191	TSC1	TSC1	CST	4906	1125	Rabbit	Use with Caution
192	TTF1	TTF1	Abcam	ab76013	1081	Rabbit	Validated
193	Tuberin	TSC2	Epitomics	1613-1	670	Rabbit	Validated
194	Tuberin_pT1462	TSC2	CST	3617	671	Rabbit	Validated
195	TYRO3	TYRO3	CST	5585	1080	Rabbit	Validated
196	UBAC1	UBAC1	Sigma Aldrich	HPA005651	1270	Rabbit	Validated
197	UGT1A	UGT1A	Santa Cruz	sc-271268	1267	Mouse	Validated
198	UQCRC2	UQCRC2	Abcam	ab14745	1256	Mouse	Use with Caution
199	VDAC1/Porin	VDAC1	Abcam	ab14734	1254	Mouse	Validated
200	VEGFR2	KDR	CST	2479	688	Rabbit	Validated
201	XRCC1	XRCC1	CST	2735	906	Rabbit	Use with Caution
202	YAP	YAP1	Santa Cruz	sc-15407	780	Rabbit	Under Evaluation
203	YAP_pS127	YAP1	CST	4911	782	Rabbit	Under Evaluation
204	YB 1	YBX1	Novus Biologicals	1725.00.02	700	Rabbit	Validated
205	YB 1_pS102	YBX1	CST	2900	835	Rabbit	Validated

Validation Status*
Valid = RPPA and WB correlation > 0.7
Use with caution = RPPA and WB correlation < 0.7
Under Evaluation = Antibody has given mixed results and / or evaluated by another lab; We are in the process of (re)validating

Preparation of tumor lysates from frozen tissue (By Precellys homogenizer) for Reverse Phase Protein Array

1. Reagents and material: Frozen tumor tissue set on dry ice, Scalper, Weighing dish, Tweezers, Lysis buffer with protease inhibitors set on ice, tubes from Precellys Ceramic Beads Kit (1.4 mm, Cat # 10011152, from Cayman Chemical, www.caymanchem.com, Phone 800-364-9897 or 734-971-3335), 1.5ml microcentrifuge tubes labeled with sample number and set on ice.

   Lysis Buffer: 1% Triton X-100, 50mM HEPES, pH 7.4, 150mM NaCl, 1.5mM $MgCl_2$, 1mM EGTA, 100mM NaF, 10mM Na pyrophosphate, 1mM Na_3VO_4, 10% glycerol, containing freshly added protease and phosphatase inhibitors from Roche Applied Science Cat. # 05056489001 and 04906837001, respectively

   4X SDS Sample Buffer: 40% Glycerol, 8% SDS, 0.25M Tris.HCL, pH 6.8. Before use, add beta-mercaptoethanol at 1/10 of volume.

2. Remove the tumor tissue from cryovials and wash out OCT medium. Cut a small piece of tumor tissue (approximately the size of a grain of rice, 20mg) and place in 2ml tubes with Ceramic Beads(for Precellys homogenizer). We can work with small volume by Precellys homogenizer. We estimate protein yield at 60ug from 1mg of tumor tissue.

3. Add ice-cold lysis buffer to the tube. The volume of lysis buffer is calculated as 40mg of tumor /ml.

4. For using Precellys, place the tubes on the rack, put the white lid on, and choose from program 1 or 2, click valid to start. Program 1 sets at 30 second per cycle for 2 cycles and Program 2 sets at 45 seconds per cycle for 2 cycles.

5. Centrifuge at 4° C for 15 minutes at maximum speed (13,000~14,000 rpm).

6. Collect supernatant (tumor lysates) and transfer to another set of microcentrifuge tubes.

7. Determine protein concentration by BCA or Bradford reaction and adjust protein concentration to 1-1.5mg/ml by lysis buffer.

8. Mix the cell lysate with 4X SDS sample buffer without Bromophenol Blue (3 parts of cell lysate plus one part of 4X SDS sample buffer). Boil the samples for 5 minutes. The samples are ready for RPPA processing. If the samples need to be stored for later use, store them in –80°C.