SUMMARY OF CHANGES: Protocol

For **Amendment #5** to: An Open Label, Multicenter, Single arm Phase II study to evaluate the Activity and Tolerability of the novel mTOR Inhibitor, MLN0128 (TAK-228), in patients with Locally Advanced or Metastatic Transitional Cell Carcinoma of the urothelial tract whose tumors harbor a *TSC1* and/or a *TSC2* mutation

NCI Protocol #: 9767
Local Protocol #: 2000021268

Protocol Version Date: 09/08/2020
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I. CTEP Request for amendment to add CATCHUP organizations:

#	Section	Comments
1.	Title Page	CATCH-UP organizations added

II. Additional Changes by Principal Investigator:

#	Section	Comments
1.	All	Updated Version Date in Header
2.	Title Page	Updated Protocol Type / Version # / Version Date

NCI Protocol #: 9767

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TITLE: An Open Label, Multicenter, Single arm Phase II study to evaluate the Activity and Tolerability of the novel mTOR Inhibitor, MLN0128 (TAK-228), in patients with Locally Advanced or Metastatic Transitional Cell Carcinoma of the urothelial tract whose tumors harbor a *TSC1* and/or a *TSC2* mutation.

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NCI-Supplied Agent: [MLN0128 (TAK-228) (MLN0128) - NSC# 768435]

Investigational Agent	**IND#**	**IND Sponsor**
MLN0128 (TAK-228)		DCTD, NCI

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SCHEMA

This is a phase II, open label, multicenter study assessing the activity and tolerability of the novel mTOR kinase inhibitor, MLN0128 (TAK-228), in patients with locally advanced or metastatic transitional cell carcinoma (TCC) of the urothelial tract harboring a *TSC1* and/or a *TSC2* mutation. The mutation must be identified by a CLIA certified lab. This protocol, as shown in the schema below, offers an optional study to perform a pre-screening test of *TSC1* and TSC2 gene sequencing for patients whose mutation status is unknown. An archival tumor sample along with buccal swab needs to be sent to Yale Clinical Molecular Pathology Laboratory (YCMPL), which serves as the Study's central lab.

For those whose *TSC* gene mutation was previously identified at a CLIA-certified lab other than YCMPL, the **case must be reviewed** by the Study PI and/or Translational PI, and the approval by the Study PI must be given for eligibility. **In addition, archival FFPE block or 10 unstained slides along with a buccal swab**, must be sent to YCMPL for confirmation of the mutation. If archival tissue is no longer available, a tumor biopsy would be required for tumor sample submission.

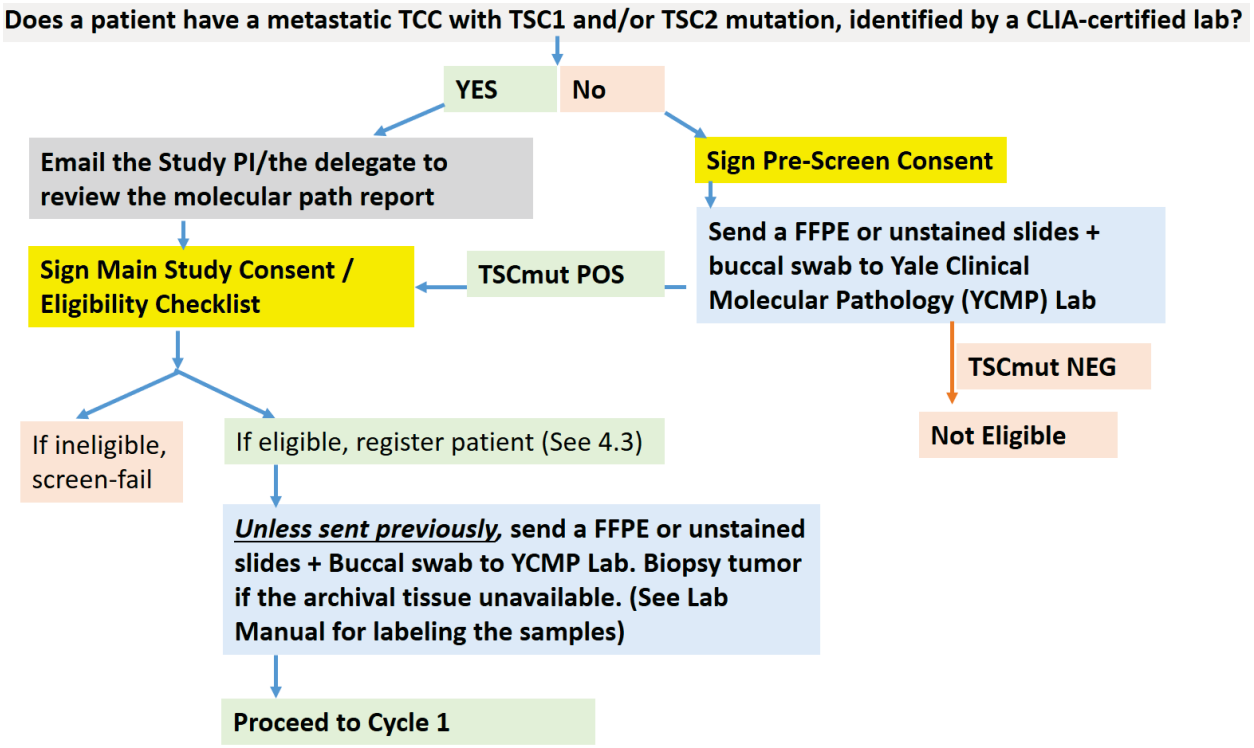

Figure 1. Flow Schema for Prescreening and Screening.

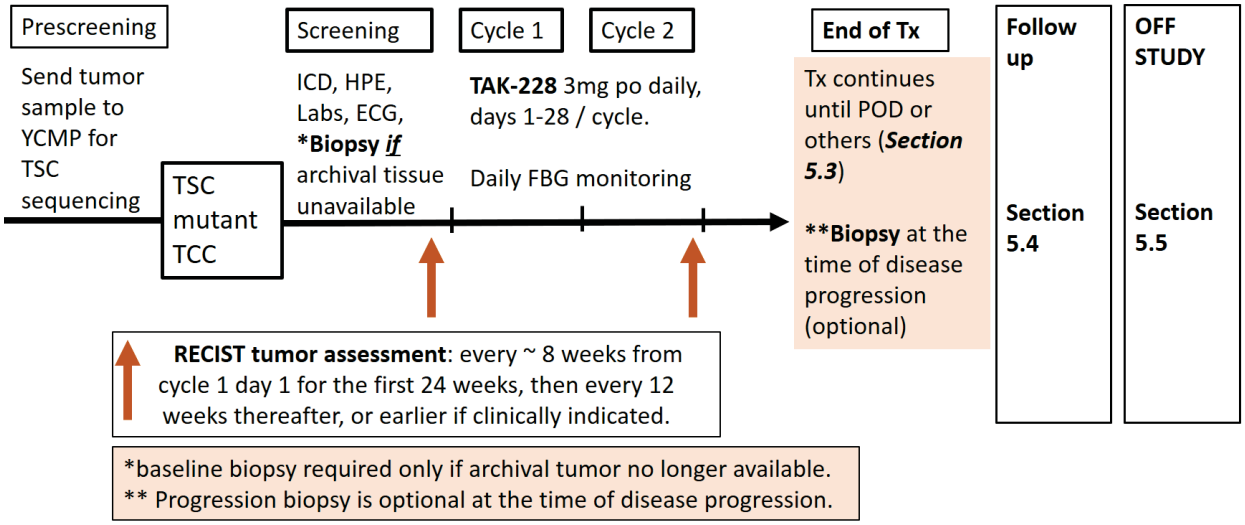

Figure 2. Diagrammatic illustration of events on clinical trial.

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LIST OF ABBREVIATIONS AND GLOSSARY OF TERMS

Common abbreviations used in oncology protocols are provided below. Program-specific or protocol-specific abbreviations must be added to this list, and unnecessary abbreviations removed, as applicable. Abbreviations that are retained should not be changed.

Abbreviation	Term
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BID	bis in die; twice a day
BUN	blood urea nitrogen
CBC	complete blood count
CLIA	Clinical Laboratory Improvement Amendments
CMS	Centers for Medicare & Medicaid Services
CYP	cytochrome P_{450}
ECOG	Eastern Cooperative Oncology Group
LDH	lactate dehydrogenase
LFT	liver function test(s)
MTD	maximum tolerated dose
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NYHA	New York Heart Association
PK	pharmacokinetic(s)
PO	*per os*; by mouth (orally)
QTc	rate-corrected QT interval (millisec) of electrocardiograph
SAE	serious adverse event

1. OBJECTIVES

1.1 Primary Objectives

- To determine the Overall Response Rate (ORR) defined as complete response (CR) and partial response (PR) in patients with locally advanced or metastatic TCC harboring a *TSC1* mutation.

1.2 Secondary Objectives

- To evaluate the safety and tolerability of MLN0128 (TAK-228) in patients with locally advanced or metastatic TCC harboring a TSC1 or TSC2 mutation.
- To evaluate progression free survival (PFS) and overall survival (OS).

1.3 Exploratory Objective

- To determine the ORR in patients with locally advanced or metastatic TCC harboring a *TSC2* mutation.
- To evaluate toxicity, PFS, and OS in *TSC2* mutation patients.

2. BACKGROUND

2.1 *Study Disease(s)*

Bladder cancer is a common malignancy. Approximately 73,000 cases of bladder cancer are diagnosed annually in the United States with more than 14,000 disease-related deaths (*Siegel et al., 2012).* TCC is the most frequent histological subtype of bladder cancer, accounting for approximately 90 percent of bladder and renal pelvis cancers. The 5-year year overall survival (OS) rate of locally advanced and metastatic TCC is approximately 35% and 5%, respectively *(Howlader et al 2012).* Cisplatin-based chemotherapy has been the standard therapeutic approach for unrespectable locally advanced and metastatic bladder cancer for the past 30 years with no standard second line therapy for progressive disease *(NCCN Guidelines 2014; Von der Maase et al 2005).* The National Comprehensive Cancer Network (NCCN) recommends participation in clinical trials of new agents for second-line therapy of metastatic disease. Exploring novel therapeutic agents in the context of relevant drugable genomic alterations represents an urgent need. Exploring novel genomic driven therapeutic approaches for this disease is an urgent need and understanding the involvement of different molecular pathways may help in developing clinical trials in a personalized fashion.

2.2 CTEP IND Agent

Second generation mTOR inhibitors are small molecule ATP-dependent TOR kinase inhibitors which target the mTOR kinase domain and are currently being investigated in clinical trials *(Schenone et al., 2011).* They are likely to suppress mTORC1 and mTORC2 functions, a feature

that could increase the antitumor effect relative to the currently available rapalogs. Those agents have been termed the TORKinibs to distinguish their mode of action from the rapalogs. They seem to inhibit protein synthesis with greater potency than rapalogs, due in part to their greater inhibition of mTORC1 action on 4EBP1 *(Wander et al., 2011).* The development of the TORKininbs has led scientists to conclude that rapalogs are ineffective at blocking cell proliferation due to their partial effect on the mTORC1, sparing mTORC2 *(Feldman and Shokat, 2010).* The profound anti-proliferative effect of the TORKinibs suggests that these molecules could be more effective in the treatment of cancers where rapalogs have failed.

MLN0128 (TAK-228), formerly known as MLN0128, is an orally available, potent, and highly selective ATP-competitive inhibitor of mTOR kinase that exhibits dual specificity against both TORC1 and TORC2 (TORC1/2) complexes *(CTEP solicitation on MLN0128; 2012*). Dual TORC1/2 inhibition mitigates the feedback activation of AKT, known to cause resistance to TORC-1 selective inhibitors such as rapamycin *(De et al., 2012).* MLN0128 (TAK-228) displayed cellular inhibition of TORC1 and TORC2 pathways with IC50 less than 10 nM. MLN0128 (TAK-228) is believed to have the potential of achieving greater clinical benefit than the currently available rapalogs. In vitro studies have demonstrated that MLN0128 (TAK-228) selectively and potently inhabits the mTOR kinases but relative to mTOR inhibition, MLN0128 (TAK-228) has >100-fold less potency as an inhibitor of Class I (PI3 kinase isoforms α, β, γ, δ), class II (PI3KC2α and PI3K2C β), and class III (VPS34) PI3K family members, as well as PI3Kα and PI3Kβ. *(Investigator's Brochure, CTEP solicitation on MLN0128, 2012).* MLN0128 (TAK-228) was also found to inhibit (>80%) the biochemical activity of five kinases (mTOR, DNA-PK, PDGFR α, Flt3, and CK1 epsilon kinases) out of a panel of 222 protein kinases. MLN0128 (TAK-228) inhibited ligand binding of 10 receptor and intracellular protein kinases including (ACVR1, BMPR1B, CSF1R, CSNK1D, CSNK1E, DDR1, MEK1, MEK2, PDGFR α, and RIPK2) out of a panel of 402 distinct kinases. In PTEN deficient cells, MLN0128 (TAK-228) had pharmacodynamic properties comparable to the dual inhibition of the TORC1 and PI3K utilizing rapamycin and PI-103 (Figure2)

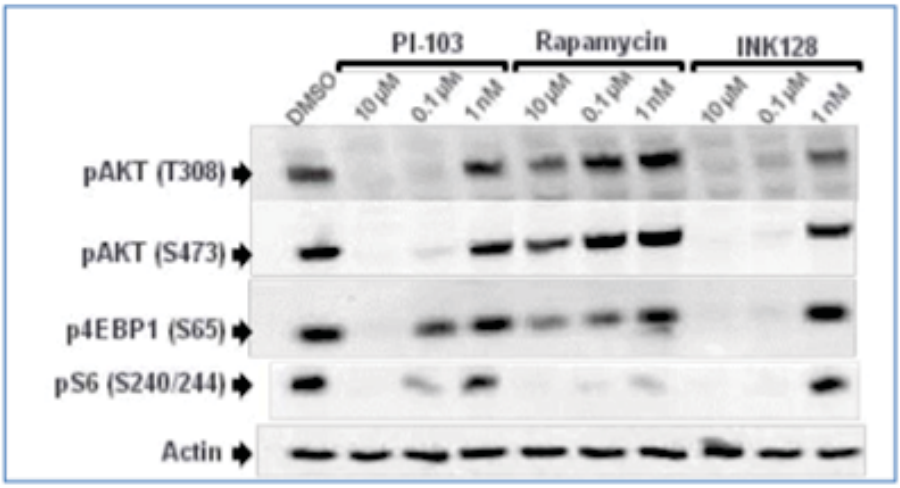

Figure 2: TAK-228 (formerly INK128 and MLN0128) modulation of TORC1 and TORC2 signaling in the PC-3 (PTEN-/-) cell line culture (Investigator's Brochure, 2012).

Legend: S6 and 4EBP1 (TORC1 targets) and AKT (TORC2 target)
TAK-228 (formerly INK128 and MLN0128), PI-103 (PI3K/mTOR inhibitor), rapamycin (TORC1 inhibitor); each agent was used at 0.001; 0.1, and 10mcM)

In Vivo Studies:

MLN0128 (TAK-228) has inhibited mTOR signaling and has demonstrated anticancer activity against a number of human solid tumor cell-line xenograft mouse models, including *PTEN* mutant endometrial (AN3-CA) and breast carcinomas (ZR-75-1), among other *PTEN* mutant carcinomas like glioblastoma and renal cell carcinoma *(Investigator's Brochure, CTEP solicitation on MLN0128, 2012).* MLN0128 (TAK-228), administered orally in multiple human tumor xenograft mouse models, inhibited angiogenesis and tumor growth by inhibiting mTOR signaling at plasma concentrations associated with *in vitro* inhibition of mTOR in a dose- and time-dependent manner and displayed a clear pharmacokinetic to pharmacodynamic (PK/PD) relationship *(Jessen et al., 2009).* MLN0128 (TAK-228) also inhibits both the phosphorylation of S6 and 4EBP1, the downstream substrates of TORC1, and selectively inhibits AKT phosphorylation at Ser473 evidenced by decrease pAKT, the downstream substrate of TORC2, *in vitro* and *in vivo*, including cell lines that are resistant to rapamycin and pan-PI3K inhibitors (*Jessen et al., 2009; Kessler et al., 2010; Hassan et al., 2013).*

Safety Pharmacology: MLN0128 (TAK-228) has a low potential to affect the human ether-a-go-go related gene (hERG) potassium ion channel and did not affect cardiovascular (CV) parameters in vivo in telemeterized monkeys.

Drug Metabolism and Pharmacokinetics: MLN0128 (TAK-228) was rapidly absorbed after PO administration to mice, rats, dogs, and monkeys,with high oral bioavailability. MLN0128 (TAK-228) displayed dose-proportional plasma exposures, a moderate propensity to cross the blood-brain barrier, and was modestly bound (70.5%) to human plasma proteins. MLN0128 (TAK-228) did not inhibit P-glycoprotein, but did inhibit breast cancer-resistance protein (BCRP), organic cation transporter (OCT)1 and OCT2.

The main isozymes responsible for phase 1 metabolism appear to be cytochrome P450 (CYP) 2C9, 2C19, and 3A4. MLN0128 (TAK-228) did not induce CYP1A2, 2B6, and 3A4 activity and expression at concentrations up to 10 μM. MLN0128 (TAK-228) displayed low potential for inhibition and is not a time-dependent inhibitor of CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4/5.

Oral administration of MLN0128 (TAK-228) 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 anticipated therapeutic dose to be used in the clinic in oncology indications (total maximum plasma concentration [C_{max}] of 0.48 μM [free C_{max} of 0.14 μM] at 30 mg once weekly [QW]).

Toxicology: Adverse events of MLN0128 (TAK-228) in rats and monkeys included body weight loss, decreased activity, increased glucose and insulin levels, alterations in white blood cells, bone marrow and lymphoid depletion, thymic necrosis, oligospermia, testes degeneration/atrophy, nonglandular stomach epithelial degeneration/ulceration/hyperplasia, pancreatic islet degeneration and fibrosis, lens fiber degeneration with cataract correlate, adrenal cortex hypertrophy, pituitary atrophy secondary to body weight loss, liver hepatocellular vacuolation, retinal dysplasia with or without optic nerve atrophy, and alveolar histiocytosis. MLN0128 (TAK-228) was negative for genotoxicity in an in vitro bacterial mutagenesis (Ames) assay, an in vivo rat micronucleus assay, and an in vivo rat comet assay. MLN0128 (TAK-228) was negative for phototoxicity in the 3T3 fibroblast assay.

Summary of Effects in Humans:

MLN0128 (TAK-228) has been studied in 2 different phase I clinical trials in patients with solid malignancies and an additional phase I trial in patients with hematologic malignancies utilizing different dosing schedules (QD, QW, QDx3d every week and QDx5d every week) in 28-day cycles *(Infante et al., 2012; Ghobrial et al., 2012; Tabernero et al., 2012)*. *The original trials with MLN0128 (TAK-228) used the original <u>unmilled</u> MLN0128 (TAK-228) active pharmaceutical ingredient (API); current manufacturing process produces <u>milled</u> MLN0128 (TAK-228) API.* The recommended phase 2 dose for unmilled MLN0128 (TAK-228) for the daily dosing schedule of the unmilled formulation were determined at 5 mg/day and for a weekly dosing schedule at 30 mg/week. *(Infante et al., 2012; Ghobrial et al., 2012).* A dose limiting toxicity of grade 3 rash was reported in in these trials *(Infante et al., 2012).*

Clinical Pharmacokinetics: MLN0128 (TAK-228) was well tolerated at the doses and schedules tested and showed a high oral bioavailability and dose-linear PK across all dosing regimens evaluated with exposures in the range of predicted biological activity *(Infante et al., 2012 and Tabernero et al., 2012).* MLN0128 (TAK-228) exhibits fast oral absorption (time to reach Cmax [tmax], generally between 1-4 hours after dosing); has dose-linear PK, with a mean plasma half-life of approximately 8 hours; and does not accumulate meaningfully in plasma when dosed as frequently as once daily (QD)

The PK of MLN0128 (TAK228) has been evaluated in over 130 patients in ongoing studies. The mean steady-state plasma C_{max} ranged from 52-232 nM. No significant change in the PK

parameters of MLN0128 (TAK228) was observed on repeat dosing; the mean accumulation index of MLN0128 (TAK228) ranged from 0.7-1.7-fold following multiple doses. PK parameters in 25 patients with advanced solid tumors receiving MLN0128 (TAK228) on the QD dosing are presented in Table 5.4 from the Investigator's Brochure v.7.

Table 5-4 Study INK128-001: Preliminary Plasma Pharmacokinetic Parameters of Multiple-Dose MLN0128 (Cycle 2, Day 1), by Dose and Regimen in Patients With Nonhematologic Malignancies

MLN0128 Regimen	C_{max} (ng/mL) Geometric Mean (CV%)	T_{max} (h) Median (min, max)	$t_{1/2}$ (h) Mean (SD)	AUC_{0-t} (ng*h/mL) Geometric Mean (CV%)	AUC_{inf} (ng*h/mL) Geometric Mean (CV%)
2 mg QD (n = 3)	15.6 (35)	2 (2, 2)	a	95.2 (46.2)	a
4 mg QD (n = 5)	20.3 (45.6)	4 (1, 4)	a	104 (54.5)	a
6 mg QD (n = 8)	40.2 (46.4)	2 (1, 4)	a	242 (51)	a
6 mg QD × 3d (n=3)	59.2 (20.7)	4 (1, 4)	8.47 (3.04)	491 (77.7)	973 (61)
7 mg QD (n = 3)	51.3 (86.9)	4 (1, 4)	a	222 (59.2)	a

Consistent with the QD dosing, MLN0128 (TAK228) displayed dose-dependent PK on all three intermittent dosing regimens, with rapid absorption (Tmax ranging from 0.5-4 hours) and mean plasma t1/2 8 hours (Tabernero *et al.*, AACR 2012).

Safety: As of the clinical data cutoff (09 December 2014), a total of 335 patients had received ≥ 1 dose of study drug across studies. A total of 18 deaths that occurred within 30 days of the last study drug dose had been reported to the clinical database as of the data cutoff; of these events, 1 (cardiac arrest; Study INK128-001) was considered related to MLN0128 (TAK-228).

At least 1 treatment-emergent SAE, regardless of causality, had been reported in 125/335 patients (37%). Across the studies and regardless of causality or dosing regimen, the most common TEAEs included nausea, fatigue, hyperglycemia, vomiting, diarrhea, stomatitis, and decreased appetite.

Due to the cardiac death on study INK128-001, study C31002, a phase 1 single-arm study to evaluate the effect of a single dose of 40 mg MLN0128 (TAK-228) on the QT/QTc interval was initiated in patients with advanced solid tumors. After completing the per-protocol PK/ECG/cardiac contractility monitoring, the patients continued MLN0128 (TAK-228) 30 mg QW with continued cardiac monitoring. The study results showed that treatment with MLN0128 (TAK-228) was not associated with clinically meaningful effects on the overall elecrocardiographic safety profile, and that ECHO/MUGA at screening was not required.

Development of a Milled Formulation of MLN0128 (TAK-228)

In order to allow more predictable absorption of MLN0128 (TAK-228) after oral administration

and to allow scale-up manufacturing of MLN0128 (TAK-228) capsules, Millenium/Takeda developed a new milled formulation of the agent. The physical milling step during the granulation process controls particle size distribution of MLN0128 (TAK-228). In order to observe whether this milling step altered the safety and PK profile of MLN0128 (TAK-228), the company performed in vivo studies with PK analysis of milled MLN0128 (TAK-228). These studies indicated that the milled formulation may result in faster absorption with possibly higher maximum concentration (Cmax), which could result in a different safety profile, compared to the previous unmilled API capsules.

Takeda developed new MLN0128 (TAK-228) capsules containing milled active pharmaceutical ingredient (API) for clinical studies in 1 mg, 3 mg, and 5 mg strengths. Patients receiving the milled formulation were added onto ongoing studies C31001 and C31002, as well as a new study MLN0128-1004, with various treatment cohorts including daily and weekly administration of milled MLN0128 (TAK-228)

The recommended dose of milled MLN0128 (TAK-228) was evaluated in 17 patients of MLN0128-1004, with PK, safety, and tolerability assessed. Six patients were given a 4 mg QD dose of milled MLN0128 (TAK-228) and 3 patients had observed DLT (rash, appetite loss and fatigue). A dose of 3 mg QD was given to 11 patients with only 1 DLT (decreased platelets) observed. The 3 mg QD dose of MLN0128 (TAK-228) was declared the RP2D, and was generally well tolerated and demonstrating objective responses in patients.

A significant and meaningful difference in tolerability was observed in the comparison of the MTDs between unmilled and milled MLN0128 (TAK-228) when administered QD. This difference in tolerability may be possibly explained due to the effect of food on the safety/tolerability of unmilled MLN0128 (TAK-228) in study IND128-001. Consequently, a dose of 3 mg QD was chosen as the RP2D of milled MLN0128 (TAK-228) dose in empty stomach conditions.

The RP2D for milled MLN0128 (TAK-228) on a weekly schedule was determined to be 30 mg, the same weekly RP2D as seen for the older unmilled formulation. Six patients treated at 30 mg weekly with the milled formulation did not demonstrate any DLT, but the agent was not escalated further. No DLT had been demonstrated for milled MLN0128 (TAK-228) at the prior 20 mg QW dose as well.

2.3 Rationale

The molecular characteristics of different tumor subtypes and the involvement of distinct pathways in tumor pathogenesis play an important role in how these tumors respond to different targeted therapies. Searching for therapeutically actionable genomic alterations and selecting patients during early drug development is being rapidly adopted to define patients who may benefit the most from these therapies. Somatic mutations and resultant molecular alterations that control the regulatory pathways of the mechanistic (formerly known as, mammalian) target of rapamycin (mTOR) complex deserve further investigation as we develop more drugs targeting this crucial pathway.

The mTOR is a complex multimolecular and multifunctional serine/threonine kinase protein that integrates a variety of incoming signals and regulates cell growth, proliferation, survival, motility

and angiogenesis *(Bjornsti et al., 2004; Chan S. 2004; Wen et al., 2013; Chiang et al., 2007).* The mTOR-signaling network involves two functionally and physically distinct and complex proteins, TORC1 and TORC2. A regulatory associated protein (Raptor) distinguishes mTORC1 complex, whereas mTORC2 contains a distinct set of protein subunits including Rictor (rapamycin-insensitive companion of mTOR), mSin1 (mammalian stress-activated protein kinase interacting protein 1), and Protor-1 subunit in place of Raptor (Figure 3) *(Guertin and Sabatini, 2007; Sabatini, 2006; Chiang et al., 2007).*

The mTORC1, stimulated by growth factors and amino acids, exerts its effects through downstream phosphorylation of p70S6 Kinase (S6K1) and 4EBP1 *(Weinberg R., 2007; Sabatini et al., 2006).* The phosphorylation of S6K1 Kinase results in its activation, which in turn phosphorylates S6 protein, an important step in 40-S ribosomal subunit formation and protein biosynthesis. Additionally, mTOR phosphorylates 4EBP1, which in turn activates eIF4E (eukaryotic Initiation Factor 4E) through forming a complex with several other initiating factors that enable the ribosomes to initiate certain mRNA translations. *(Weinberg R., 2007; Sabatini et al., 2006).*

There are several critical upstream regulators of the mTOR pathways *(Fingar et al., 2004).* The phosphatidylinositol 3'-kinases (PI3K) are lipid kinases that exert their function by the production of the second messenger phosphatidylinositol 3,4,5-triphosphate (PIP3), which in turn contributes to the recruitment and activation of a wide range of down-stream targets, including Akt (also known as protein kinase B", PKB) *(Stokoe et al., 1997).* PTEN (phosphatase and tensin homolog deleted on chromosome ten) is a lipid phosphatase that counteracts the activity of PI3K by dephosphorylating PIP3 *(Stambolic et al., 1998).* Activation of AKT/PKB results in increased cell growth and proliferation, a shift to glycolytic metabolism, and increased cell migration in addition to its anti-apoptotic activity. *(Bhaskar et al., 2007; Sabassov et al., 2005).* AKT/PKB exerts its effect through phosphorylation and inactivation of the mTOR-inhibitory tuberous sclerosis (TSC) complex formed by hamartin and tuberin (also called TSC1 and TSC2 respectively) (*Bjornsti et al., 2004; Weinberg R., 2007; Chiang et al., 2007).* TSC is a GTPase activating protein (GAP) that exerts its effect through binding to the Rheb (Ras homolog enriched in brain) forming a complex (Rheb-GTPase) that directly binds to the mTORC1 and inhibit downstream effects of the mTOR pathway. As a Rheb specific GAP, the TSC complex acts as a signal-modulated suppressor of Rheb by driving Rheb into the inactive, GDP-bound state and thus prevents Rheb-GTP mediated mTORC1 activation. *(Chiang et al., 2007; Tee et al., 2003; Long et al., 2005).* AKT also phosphorylates and functionally inhibits TSC2, which indirectly activates the mTORC1. *(Potter et al., 2002; Manning et al., 2002; Inoki et al., 2002).* Evidence suggests that a loss of function mutation in *PTEN, TSC1* and *TSC2* results in constitutive activation of the mTORC1 pathway *(Mehran et al., 2001; Magri et al., 2011)* with persistent phosphorylation of the p70S6K (S6K) and its substrate S6 that is sensitive to pharmacologic inhibition` of the mTOR. *(Kwiatkowski et al., 2002; Inoki et al., 2009; Zhang et al., 2003; Mehran et al., 2001).*

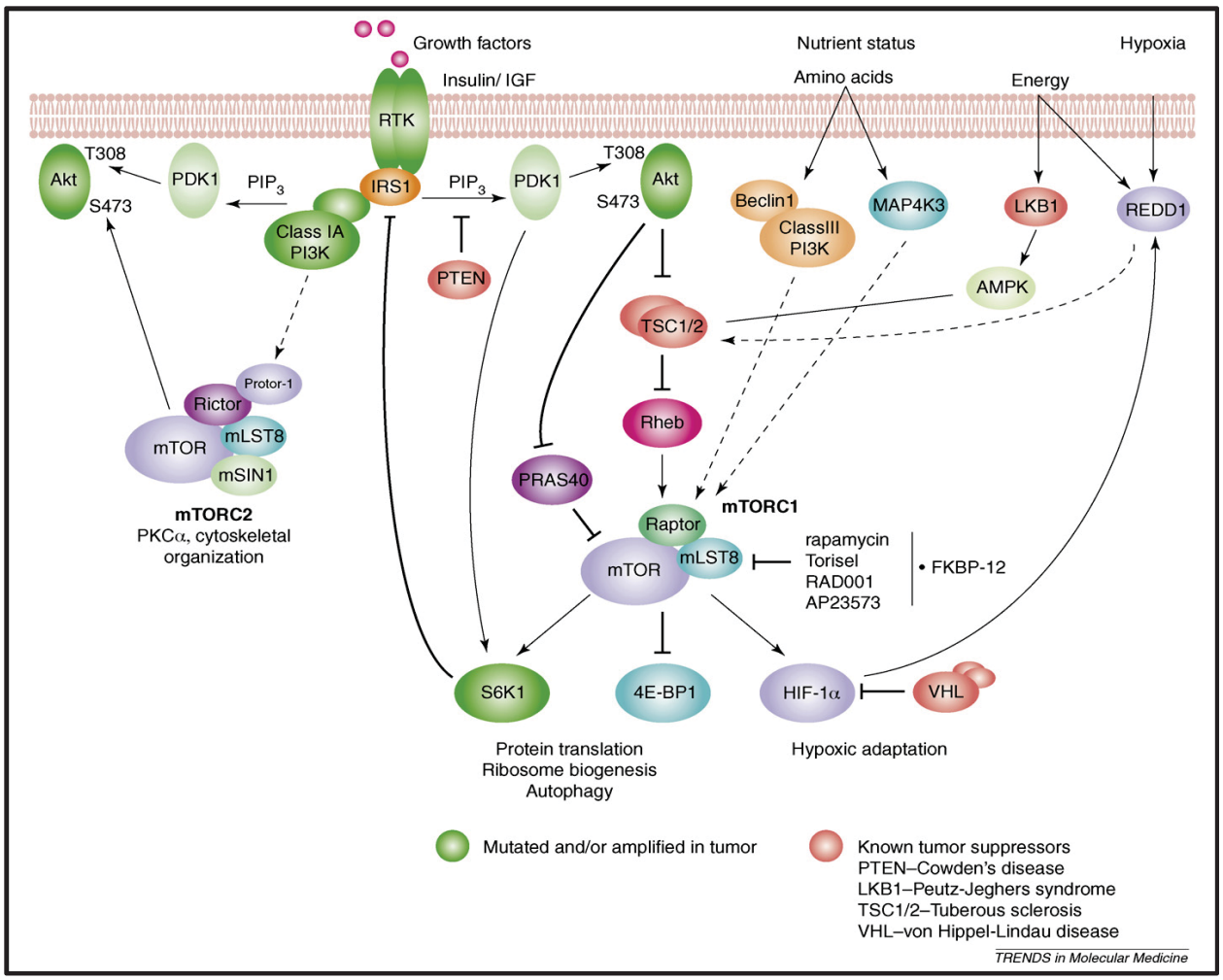

Figure 3. The mTOR-signaling network. Adapted from *Chiang G, Abraham R. Trends Mol Med 2007*

To add to the complexity of this network, it is important to recognize that mTORC1 is a downstream recipient of AKT dependent stimulatory signals while mTORC2 is an upstream activator of AKT. In other words, mTORC2 phosphorylates AKT/PKB, which is an oncogenic regulator of apoptosis and proliferation *(Weinberg R., 2007; Sarbassov et al., 2005; Chiang et al., 2007).* So, inhibiting both TORC complexes is important to gain better control over this pathway. Additionally, there are other known proteins that regulate the mTOR pathways include FBXW7. The latter is a protein product of the tumor suppressor gene, *FBXW7*, which binds and targets the mTOR for ubiquitination and consequent degradation *(Mao et al. 2008)*. Somatic mutations that result in loss of function of FBXW7 occur in approximately 6% of human cancers and are believed to result in constitutive activation of the mTOR complex. *(Akhoondi et al., 2007; Mao et al., 2008)*

Targeting the mTOR-signaling pathway has already proven itself in the clinic; however, with limited success. The first generation mTOR inhibitors such as rapamycin and its analogs (everolimus, temsirulimus, ridaforolimus) are called the rapalogs. They are allosteric modulators of the TORC1 complex that exhibited limited anticancer activity through downstream inhibition of phosphorylation of p70S6 Kinase (S6K1) and 4EBP1 *(Weinberg R., 2007;* Sabatini et al., 2006*)*.

One of the limitations of these drugs that may explain their limited success in the clinic is that they selectively inhibit TORC1 without affecting TORC2 failing to inhibit all mTOR functions. Also, mTOR may influence PI3K signaling *via* the S6K-IRS1 feedback loop and *via* mTORC2-mediated phosphorylation of AKT at serine 473 *(Chiang et al., 2007; Markman et al., 2010)*. Finally, resistance to TORC1 inhibition by rapalogs may develop from activation of the MAPK pathway *(Carracedo et al., 2008)*. Inhibition of TORC2 has been shown to reverse the AKT activation induced by TORC1 inhibition *(Nardella et al., 2009)*.

Several components of the PI3K/Akt/mTOR pathway can be altered during the pathogenesis of TCC, which results in uncontrolled cell proliferation. *(Garcia et al., 2008; Chen et al., 2010)*. Genomic alterations in three of the key genes in the mTOR pathway (*PIK3CA, TSC1/2,* and *PTEN*) are not mutually exclusive in TCC but they play a major role in upstream regulation of this pathway *(Platt et al., 2009)*. As previously mentioned, loss of function mutation in the *TSC1* and *TSC2* have been shown to activate the mTORC1 pathway constitutively *(Magri et al., 2011)*. *TSC1* is a tumor suppressor gene that is involved in the pathogenesis of TCC and loss of heterozygosity (LOH) involving the *TSC1* locus at 9q34 occurs in more than 50% of these tumors. Several genetic alterations of *TSC1* have been identified in 8-16% of TCCs *(Hornigold et al., 1999; Habuchi et al., 1995; Platt et al., 2009, TCGA bladder cancer 2014)*. The most common alterations described in TSC1 and TSC2 bladder cancers are inactivating Copy Number Alterations (CAN) and truncating mutations (*Iyer et al., 2012; TCGA bladder cancer 2014)*. The inactivating somatic mutations of *TSC1* gene in TCC seem to be, in part, different from the mutation spectrum seen in tuberous sclerosis syndrome *(Knowles et al., 2003; Pymar et al., 2008)*. LOH involving the *TSC2* locus at 16q13.3 has been described in about 15% of TCCs with array based CGH analysis showing under-representation of *TSC2* region in 9.7% *(Platt et al., 2009)*. TSC1, as well as TSC2, were reported to be mutated with combined frequency of 15% in bladder cancer in one study *(Sjödahl et al 2011)*.

As many components of the mTOR upstream regulatory pathways are dysregulated in urothelial TCC, an agent or multiple agents that can potentially target these pathways may be the best strategy to control this disease. The successful use of rapalogs in the treatment of subependymal giant-cell astrocytoma in patients with tuberous sclerosis suggested that mTOR inhibitors could be a therapeutic option for tumors that harbor a *TSC* mutation. *(Franz et al., 2006; Krueger et al., 2010)*. Everolimus has demonstrated a clinical benefit in TCC patients treated on a phase II clinical trial. PFS of 3.3 months and an OS of 10.5 months were reported (*Seront et al., 2012)*. Further analysis of tumor samples of patients on this trial utilizing Whole Genome Sequencing (WGS) identified inactivating mutations in *TSC1* as a biomarker of clinical benefit. Examples of these mutations included R629*; R509*; Q694*; V220F; E636fs (*Iyer et al., 2012)*. The majority of patients with *TSC1* wild type tumors who were treated with everolimus progressed within 2 months of therapy, while patients whose tumors were *TSC1* mutant remained on therapy for 7.7 months (p=0.004) *(Iyer et al., 2012)*. This trial looked only at the relevance of TORC1 inhibition in relation to everolimus response in a retrospective manner. The relevance of TSC2 mutations in relation to everolimus sensitivity has not been studied. These results suggest that utilizing mTOR inhibitors for *TSC1* mutated TCC patients is a rational approach that should be further explored in the context of a genomic driven clinical trial. MLN0128 (TAK-228) has demonstrated a broader range of activity and this may translate into additional benefit when using MLN0128 (TAK-228) in TSC1/2 mutated TCC. Dual TORC inhibition in addition to the wide range of activity of MLN0128 (TAK-228) may help to mitigate possible resistance mechanisms that may develop during the course of

treatment.

It is unknown if the biologic and clinical effects of *TSC1* and *TSC2* mutations in TCC are identical but both TSC1 and TSC2 are upstream regulators of the mTOR pathway. Utilizing NGS will help identify patients with *TSC1* and/or *TSC 2* mutated tumors who may benefit the most from MLN0128 (TAK-228) and shed more light on the complex genomic machinery of mTOR regulation.

Studying the antitumor efficacy and safety of MLN0128 (TAK-228) in the context of a genomic driven clinical trial in TCC is warranted with the potential that MLN0128 (TAK-228) could be more effective in the treatment of cancers where rapalogs have demonstrated limited activity.

2.4 Correlative Studies Background

This study will be using an integral biomarker by identifying bladder cancer patients whose tumors harbor a *TSC1* and/or *TSC2* mutation identified by a CLIA-certified laboratory. If *TSC1* and/or *TSC2* mutation unknown, this protocol will pre-screen patients with locally advanced or metastatic TCC for *TSC1* and/or *TSC2* mutation. It will require submission of tumor tissue in a form of 10 unstained slides, or fresh frozen, paraffin embedded (FFPE) block to the CLIA-certified Molecular Profiling Laboratory at Yale University under the direction of Dr. Jeffrey Sklar. The TSC1/TSC2 test panel is described in Section 9.1.

Only those patients who harbor mutations in the *TSC1* and/or *TSC2* and meet other eligibility criteria will be treated with MLN0128 (TAK-228). It is unknown if the biologic and clinical effects of *TSC1* and *TSC2* mutations in TCC are identical. The available literature supports a positive correlation between *TSC1* mutation and clinical benefit with rapalog treatment; however, it is unknown if the same benefit is possible with *TSC2* mutated TCC. The primary objective will be to study the activity of MLN0128 (TAK-228) in *TSC1* mutated TCC. We will also evaluate the activity and tolerability of MLN0128 (TAK-228) in *TSC2* mutated TCC in an exploratory fashion to gain experience as we accrue in the TSC1 cohort to meet its accrual objectives of evaluable patients.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have measurable disease as defined by RECIST version 1.1. See Section 11 for the evaluation of measurable disease.

3.1.2 Must have a histologically confirmed transitional cell carcinoma (TCC, also known as urothelial carcinoma), locally advanced or metastatic.

3.1.3 Patient must have TCCs tumors harboring a *TSC1* or *TSC2* mutation identified by a CLIA certified laboratory.

3.1.4 Unless the prescreening was performed at Yale Clinical Molecular Pathology Lab (YCMPL), patients must have TCC tumor tissue available for submission in a form of at least 10 unstained slides or FFPE block (FFPE block highly recommended and preferred) along with a buccal swab (See 9.1 for tissue submission procedure). If the number of slides is less than 10, a biopsy should be considered. If a biopsy is deemed unsafe, the case may be discussed with the Study PI and approval must be given for eligibility.

3.1.5 Patient must have developed disease progression during or following treatment with at least one platinum-containing regimen (e.g., GC, MVAC, CarboGem) for inoperable locally advanced or metastatic urothelial carcinoma or disease recurrence, or must be unfit or ineligible for cisplatin-based chemotherapy as defined below. There is no restriction on the number of prior lines of chemotherapeutics agents received.

- Patients who progressed within 12 months of treatment with a platinum-containing neoadjuvant or adjuvant regimen are considered second-line patients. Therefore, these patients may be also eligible.

- Patients who are unfit or ineligible for cisplatin-based chemotherapy as defined by any one of the following criteria are eligible for this trial:
  a. ECOG performance score of 2
  b. Creatinine clearance < 60 mL/min
  c. A hearing loss (measured by audiometry) of 25 dB at two contiguous frequencies
  d. Grade ≥ 2 peripheral neuropathy

3.1.6 Age ≥18 years.
Because no dosing or adverse event data are currently available on the use of MLN0128 (TAK-228) in patients <18 years of age, children are excluded from this study.

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

3.1.8 Life expectancy of greater than 12 weeks.

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

– Hemoglobin	≥ 9g/dL
– Fasting serum glucose	≤ 130 mg/dL
– HbA1c	<7.0%
– Fasting triglycerides	≤ 300 mg/dL
– Leukocytes	≥3,000/mcL
– Absolute neutrophil count	≥1,500/mcL

–	Platelets	≥100,000/mcL
–	Total bilirubin	within normal institutional limits
–	AST (SGOT)/ALT (SGPT)	≤ 2.5 × institutional upper limit of normal (ULN) and ≤ 5 ULN if liver metastases are present.
–	Creatinine	≤ 1.5 x upper normal institutional limits (UNL) OR
–	Creatinine clearance	≥ 40 mL/min based either on Cockroft-Gault estimate or based on urine collection (12 or 24 hour)

3.1.10 Patients with controlled Diabetes are allowed on study. Controlled diabetes is defined as FBS 130 mg/dL or less, and patients whose FBS can be brought in this range with medical therapy are eligible for trial inclusion.

3.1.11 The effects of MLN0128 (TAK-228) on the developing human fetus are unknown. Fertility and developmental studies with MLN0128 (TAK-228) have not been conducted. On the basis of potential hazard of other mTOR inhibitors (i.e., rapamycin and other rapalogs) on the developing fetus, women of childbearing age should avoid becoming pregnant while taking any mTOR inhibitor including MLN0128 (TAK-228).

Female patients must:

- be postmenopausal for at least 1 year before the screening visit, OR
- be surgically sterile, OR
- if they are of childbearing potential, agree to practice **1 highly effective method** of contraception and **1 additional effective (barrier) method**, at the same time, from the time of signing the informed consent through **90 days** (or longer, as mandated by local labeling [e.g., USPI, SmPC, etc.;]) after the last dose of study drug, OR
- agree to practice **true abstinence,** when this is in line with the preferred and usual lifestyle of the patient. NOTE: **Periodic abstinence** [e.g., calendar, ovulation, symptothermal, postovulation methods], **withdrawal, spermicides only, and lactational amenorrhea are not acceptable** methods of contraception. Female and male condoms should not be used together.

Male patients, even if surgically sterilized (i.e., status postvasectomy), must

- agree to **practice highly effective** barrier contraception during the entire study treatment period and through **120 days** after the last dose of study drug, **OR**
- agree to practice **true abstinence**, when this is in line with the preferred and usual lifestyle of the patient (NOTE: Periodic abstinence [e.g., calendar, ovulation, symptothermal, postovulation methods for the female partner], withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception. Female and male condoms should not be used together).
- **AND** agree not to donate sperm during the course of this study or within 120 days after receiving their last dose of study drug.

3.1.12 Ability to swallow oral medications.

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

3.2 Exclusion Criteria

3.2.1 Patients who have had chemotherapy, immunotherapy, or investigational therapy, within 4 weeks (6 weeks for nitrosoureas or mitomycin C), or palliative radiotherapy within 2 weeks prior to the first dose of the study drug..

3.2.2 Patients who have not recovered from adverse events due to prior anti-cancer therapy to grade 1 or baseline. Patients with stable, controlled grade 2 AEs such as peripheral neuropathy, hypothyroidism, hypertension, adrenal insufficiency or alopecia are allowed after discussing with the PI.

3.2.3 Patients with known symptomatic, untreated central nervous system (including brain, spinal cord). Patients who have a history of brain/CNS metastasis are eligible for the study provided that all the following criteria are met:

a) Brain/CNS metastases which have been treated
b) No evidence of disease progression for ≥3 months before the first dose of study drug.
c) No hemorrhage after treatment
d) Off-treatment with dexamethasone for 4 weeks before administration of the first dose of TAK-228
e) No ongoing requirement for dexamethasone or anti-epileptic drugs

3.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to MLN0128 (TAK-228).

3.2.5 Subjects who are on systemic corticosteroids (IV or oral steroids, excluding inhaled, topical or ophthalmic corticosteroids), or anti-epileptic drugs for treated brain metastasis.

3.2.6 Subjects taking strong inhibitors and/or inducers of cytochrome P450 (CYP) 3A4, CYP2C19 or CYP2C9 within 1 week preceding the first dose of MLN0128 (TAK-228).

If a subject requires treatment with strong inhibitors and/or inducers of CYP3A4, CYP2C19 and/or CYP2C9, alternative treatment must be considered. If no alternative is available, one such medication may be allowed after discussing with the Study PI. (See Appendix F).

3.2.7 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia (excluding rate controlled atrial fibrillation/flutter), or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.8 Pregnant and breastfeeding women are excluded from this study because fertility and developmental studies with MLN0128 (TAK-228) have not been conducted and there is a potential risk for adverse events including teratogenicity and risk of abortion. Breastfeeding should be discontinued if the mother is treated with MLN0128 (TAK-228).

3.2.9 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with MLN0128 (TAK-228); however, HIV patients treated with regimens that have low CYP450 inhibition may be allowed as long as the patient's general health and CD4 counts are within acceptable levels.

3.2.10 Patients with baseline prolongation of the rate-corrected QT interval (QTc) (e.g., repeated demonstration of QTc interval > 480 milliseconds, or history of congenital long QT syndrome, or torsades de pointes).

3.2.11 Patients with untreated or active Hepatitis B or C infection.

3.2.12 Significant active cardiovascular or pulmonary disease at the time of study entry including

A. Uncontrolled high blood pressure (i.e., systolic blood pressure >180 mm Hg, diastolic blood pressure > 95 mm Hg)
B. Pulmonary hypertension
C. Uncontrolled asthma or O2 saturation < 90% by ABG (Arterial Blood Gas) analysis or pulse oximetry on room air
D. Significant valvular disease; severe regurgitation or stenosis by imaging independent of symptom control with medical intervention, or history of valve replacement
E. Medically significant (symptomatic) bradycardia

3.2.13 Manifestations of malabsorption due to prior gastrointestinal (GI) surgery, GI disease, or for an unknown reason that may alter the absorption of MLN0128 (TAK-228).

3.2.14 Concomitant administration of any proton pump inhibitor (PPI) is not permitted during the study. Patients receiving PPI therapy before enrollment must stop using the PPI for 7 days before their first dose of study drugs.

3.2.15 History of any of the following within the last 6 months prior to study entry:

A. Ischemic myocardial event, including angina requiring therapy and artery revascularization procedures
B. Ischemic cerebrovascular event, including TIA and artery revascularization procedures
C. Requirement for inotropic support (excluding digoxin) or serious (uncontrolled) cardiac arrhythmia (including atrial flutter/fibrillation, ventricular fibrillation or ventricular tachycardia)
D. Pulmonary embolism
E. New York Heart Association (NYHA) Class III or IV heart failure

F. Placement of a pacemaker for control of rhythm

3.2.16 Subjects who have initiated treatment with bisphosphonates less than 30 days prior to the first administration of MLN0128 (TAK-228). Concurrent bisphosphonate use is only allowed if the bisphosphonate was initiated at least 30 days prior to the first administration of MLN0128 (TAK-228).

3.2.17 Patients who received prior PI3K, AKT or mTOR inhibitors are not allowed.

3.2.18 Patients who received radiation therapy within the last 4 weeks. Radiation exposure may not exceed 30% of marrow area.

4. REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN or RAVE or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✔	✔		
Financial Disclosure Form	✔	✔	✔	
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔	
HSP/GCP training	✔	✔	✔	
Agent Shipment Form (if applicable)	✔			
CV (optional)	✔	✔	✔	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and

NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval
- Assigned the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR Help Desk by email at < RCRHelpDesk@nih.gov>

4.2 Site Registration Process

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to the following:

- An active Federal Wide Assurance (FWA) number
- An active roster affiliation with the Lead Network or a participating organization
- A valid IRB approval
- Compliance with all protocol specific requirements

In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:

- Active registration status
- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site.

Sites participating on the NCI CIRB initiative that are approved by the CIRB for this study are not required to submit IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRBManager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

4.2.1 Downloading Regulatory Documents

Site registration forms may be downloaded from the NCI#9767 protocol page located on the CTSU Web site. Permission to view and download this protocol is restricted and is based on person and site roster data housed in the CTSU RSS. To participate, Investigators and Associates must be associated with the Corresponding or Participating protocol organization in the RSS.

- Go to https://www.ctsu.org and log in using your CTEP IAM username and password.
- Click on the Protocols tab in the upper left of your screen.
- Either enter the protocol # in the search field at the top of the protocol tree, or
- Click on the By Lead Organization folder to expand, then select LAO-CT018, and protocol #9767.
- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided. (Note: For sites under the CIRB initiative, IRB data will load to RSS as described above.)

4.2.2 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab →Regulatory Submission
When applicable, original documents should be mailed to:
CTSU Regulatory Office
1818 Market Street, Suite 3000
Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

4.2.3 Requirements For Protocol #9767 Site Registration:

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)

4.2.4 Checking **Site** Registration Status

You can verify your site registration status on the members' section of the CTSU website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM

username and password

- Click on the Regulatory tab at the top of your screen
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements as outlined by the Lead Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

4.3 Patient Registration

4.3.1 OPEN / IWRS

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available to users on a 24/7 basis. It is integrated with the CTSU Enterprise System for regulatory and roster data interchange and with the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. Patient enrollment data entered by Registrars in OPEN / IWRS will automatically transfer to the NCI's clinical data management system, Medidata Rave.

The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

This trial uses slot reservation. For this study, OPEN will connect to IWRS at enrollment initiation to check slot availability. **Registration staff should ensure that a slot is available and secured for the patient before completing an enrollment.**

IMPORANT NOTE: Patient's slot reservation must be done **first before sending the samples for TSC pre-screening test**. The Study PI or his delegate will be in charge of approving the slot reservation request.

4.3.2 OPEN/IWRS User Requirements

OPEN/IWRS users must meet the following requirements:

- Have a valid CTEP-IAM account (*i.e.*, CTEP username and password).
- To enroll patients: Be on an ETCTN Corresponding or Participating Organization roster with the role of Registrar. Registrars must hold a minimum of an AP registration type. If a DTL is required for this study, the registrar(s) must also be assigned the OPEN Registrar task on the DTL.
- Have regulatory approval for the conduct of the study at their site.

Prior to accessing OPEN/IWRS, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.
- If applicable, all patients have signed an appropriate consent form and HIPAA authorization form.

4.3.3 OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN tab of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Theradex has developed a Slot Reservations and Cohort Management User Guide, which is available on the Theradex website: http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk at 609-619-7862 or Theradex main number 609-799-7580; CTMSSupport@theradex.com

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 7 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

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. To ensure compliance, patients will be requested to maintain a medication diary of each dose of medication (Appendix D). The medication diary will be returned to clinic staff at the end of each course.

The starting dose of MLN0128 (TAK-228) is 3mg by mouth once a day. One cycle consists of 28 calendar days.

Dose Level	***MLN0128 (TAK-228)* Dose**
-3	1 mg 5 days per week
-2	2 mg daily for 5 days out of 7
-1	*2 mg, daily*
1	*3 mg, daily*
Level 1 is the starting dose.	

MLN0128 (TAK-228) should be taken on an empty stomach at least 2 hours after food and do not eat or drink (except water) for at least one hour after taking MLN0128 (TAK-228). Do not chew, open or manipulate the capsule in any way prior to swallowing. Each dose should be taken with

8 ounces (240 mL) of water). 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 (subjects should not take 2 consecutive daily doses within 12 hours of each other). Subjects who vomit shortly after receiving MLN0128 (TAK-228) will not receive a replacement dose. If confirmed that the study drug has been vomited, the dose should be noted as having been missed."

There is a potential for interaction of MLN0128 (TAK-228) with other concomitantly administered drugs through the cytochrome P450 system. MLN0128 (TAK-228) displayed low potential (IC_{50} > 25 μM) for inhibition of the major human CYP isoforms. Although potential drug-drug interactions with MLN0128 (TAK-228) cannot be ruled out based on the known metabolism characteristics of MLN0128 (TAK-228), the potential risk is considered low. Patients receiving any medications or substances that are inhibitors or inducers of can be treated with caution. (See Appendix F).

If severe emesis or mucositis prevents the patient from taking scheduled doses, that dose will be skipped. If emesis occurs after study medication ingestion, the dose will not be readministered, and patients should resume dosing at the next scheduled time with the prescribed dosage. Patients should record the occurrence of the emesis in their dosing diaries. Under no circumstance should a patient repeat a dose or double-up doses.

5.2 General Concomitant Medication and Supportive Care Guidelines

As there is a potential for interaction of MLN0128 (TAK-228) with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies.

The following medications and procedures are prohibited during the study:

- Other investigational agents or mTOR inhibitors.
- Other anticancer therapies including chemotherapy, immunotherapy, radioimmunotherapy, targeted agents, radiation or surgery (subjects can have palliative radiation or surgery in the study for pre-existing lesions)
- Systemic corticosteroids (either IV or oral steroids, excluding inhalers) unless necessary for treatment of MLN0128 (TAK-228) related AE, ie, rash
- Anti-epileptic drugs for subjects with treated brain metastasis
- Strong CYP3A4/CYP2C19 inducers/inhibitors (See Appendix F). If a subject requires treatment with 1 or more of the strong CYP3A4 and CYP2C19 inhibitors and/or inducers, the study doctor should be consulted.
- Concomitant proton pump inhibitors, due to effects on MLN0128 (TAK-228) absorption.

There are no known strong specific CYP2C9 inhibitors or inducers. Examples of moderate inhibitors of CYP2C9 are fluconazole and miconazole; and moderate inducers 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. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with

the potential to affect selected CYP450 isoenzymes. Appendix C presents guidelines for identifying medications/substances that could potentially interact with the study agent(s).

Subjects should not consume food or beverages containing the fruit or juice from grapefruits or Seville oranges within 7 days before first dose of study drug and throughout the study.

Prophylactic use of anti-emetic, anti-nausea, and antidiarrheal medications are encouraged and may be used prior to first dose of study drug (MLN0128 (TAK-228)), and as needed throughout the study prior to each dosing and as clinically indicated per standard practice. Initiation of hematopoietic growth factors, transfusions of blood, and blood products should not be used in the first cycle unless absolutely clinically necessary. However, they may be administered after Cycle 1 if needed. Subjects who have been on chronic erythropoietin for ≥ 30 days may continue to receive the concomitant medication upon study entry.

Concurrent bisphosphonate use is only allowed if the bisphosphonate was initiated at least 30 days prior to the first administration of MLN0128 (TAK-228). Bisphosphonates should be given after Cycle 1 to minimize confounding factors which may contribute to potential drug related toxicities.

5.3 Duration of Therapy

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

- Disease progression,
- 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.

5.4 Duration of Follow Up

- Patients who discontinued the study treatment due to disease progression will be followed for 4 weeks after the last dose of the study drug or until the resolution or stabilization of treatment-related adverse events.
- Patients who discontinued the study treatment for reasons other than disease progression or death will be followed with the restaging scans as clinically indicated until disease progression, or initiation of the subsequent therapy is documented.

- Patients who discontinue the study treatment due to unacceptable treatment related toxicity will be followed until resolution or stabilization of the treatment related adverse event.

- All participants' vital status may be followed after removal from the study treatment via a phone call or medical records review every 6 months and using publicly available databases.

5.5 Criteria for Removal from Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 Special Warnings and Special Precautions for Use

Insulin and Glucose Levels
Hyperglycemia and hyperinsulinemia are known toxicities associated with inhibition of mTOR (mechanistic [formerly mammalian] target of rapamycin) and related pathways based on nonclinical studies.

A rise in fasting plasma glucose has been observed as early as 1 to 2 days following oral administration of MLN0128 (TAK-228). Early initiation of treatment of the hyperglycemia are essential. Subjects with Grade 1 hyperglycemia (fasting serum glucose [FSG] > the upper limit of the normal range ≤ 160 mg/dL) are treated with oral hypoglycemic agents (eg, metformin), and subjects with ≥ Grade 2 hyperglycemia (FSG > 160 mg/dL) are treated aggressively with oral hypoglycemic agents and/or insulin as clinically indicated. Early treatment, as noted previously, have resulted in good control of glucose levels for the majority of MLN0128 (TAK-228)-treated subjects who developed hyperglycemia.

Cardiac Effects
Cardiac events (including QT interval corrected for heart rate prolongation and arrhythmias) have been infrequently observed in clinical studies of MLN0128 (TAK-228). To date, there has been 1 report of ventricular fibrillation and cardiac arrest postdose that had a fatal outcome and was assessed as possibly related to MLN0128 (TAK-228). Routine cardiac monitoring with baseline electrocardiogram (ECG) or multigated acquisition scan and on-study ECGs and physical examination constitute the core cardiac safety monitoring in all MLN0128 (TAK-228) studies.

For subjects showing any signs of cardiac instability after MLN0128 (TAK-228) dosing, additional monitoring onsite before clinic discharge should be considered.

Renal Function
Grade 1 and 2 elevations in creatinine (regardless of causality) have been observed in subjects receiving MLN0128 (TAK-228), all of which have been reversible with drug interruption and/or supportive care with intravenous (IV) hydration. Further evaluation of the renal insufficiency with urine electrolytes suggested a pre-renal etiology with a low fractional excretion of sodium < 1%. However, the adverse event cases were confounded by multiple factors such as nausea, vomiting, hyperglycemia, concomitant medications with GI side effects such as metformin, and hydronephrosis, any of which may have also contributed to dehydration and elevated creatinine. Subjects should be encouraged to drink at least 20 ounces of fluids a day, especially on days requiring fasting (per protocol), with administration of IV fluids in the clinic as indicated to avoid dehydration.

Baseline macroscopic urinalysis and routine serum chemistries along with other safety laboratory assessments are performed in all MLN0128 (TAK-228) studies. Additionally, microscopic urinalysis, a 12-hour urine collection, spot urine electrolytes, protein and creatinine, and serum chemistry should be collected at any time when the serum creatinine is ≥ Grade 1, according to National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0, to further evaluate possible etiologies for the renal dysfunction.

Rash
Rash observed in clinical studies of MLN0128 (TAK-228) tends to be maculopapular and pruritic and has ranged from Grade 1 to 3. For the most part, rash and pruritus improve with antihistamines, topical steroid creams, and/or dose interruption. Some subjects have required pulse systemic steroids, dose reduction, and/or study treatment discontinuation.

Pneumonitis
Early recognition, prompt intervention, and a conservative risk management approach are recommended due to pneumonitis that has been observed with rapalog therapy. While no cases of pneumonitis assessed as related to MLN0128 (TAK-228) have been reported, this event will be closely monitored in all MLN0128 (TAK-228) study subjects.

Abdominal Pain/Pancreatitis
Abdominal pain and radiologic and biochemical evidence of pancreatitis have recently been seen in two patients treated with MLN0128 (TAK-228) and aflibercept. While this may not be a problem on single-agent MLN0128 (TAK-228), amylase and lipase tests should be performed for any patient on trial with unexplained abdominal pain.

If the patient develops a toxicity that requires treatment delays or dose modifications, the following dose de-escalation schema is suggested:

Dose Level	***MLN0128 (TAK-228)* Dose**
-3	1 mg 5 days per week
-2	2 mg daily for 5 days out of 7
-1	*2 mg, daily*

1 (RP2D)	*3 mg, daily*
Level 1 is the starting dose.	

6.2 Dose Modification Guidelines:

MLN0128 (TAK-228) dosing should be withheld for any ≥ Grade 3 MLN0128 (TAK-228)-related toxicities. If the event resolves to Grade ≤ 1 or baseline values within 14 days of interrupting therapy, subject may resume study treatment at one dose level lower.

In general, if MLN0128 (TAK-228) dosing is delayed for > 14 consecutive days for MLN0128 (TAK-228)-related toxicity despite supportive treatment per standard clinical practice or more than 3 dose reductions of MLN0128 (TAK-228) is required in a subject, stop MLN0128 (TAK-228) therapy, discontinue the subject from the study, and complete the EOT visit within30-40 days of the last administration of MLN0128 (TAK-228).

As a general principle, grade 4 MLN0128 (TAK-228)-related toxicities of any duration require permanent discontinuation of the study drug with the following the exception:

- Clinically insignificant lab abnormalities that resolve within two days on optimum treatment.

See the Tables below for specific guidelines, which overrides the aforementioned general principle.

The following tables represent some modification guidelines tables for some possible adverse events:

6.2.1 Management of Nausea and/or Vomiting

Nausea and/or Vomiting			
Grade	**Description**	**Treatment**	**MLN0128 (TAK-228) Dose Modification**
≤ 2	Loss of appetite with or without decreased oral intake; 1-5 episodes of vomiting within 24 hours	Maximize anti-emetic therapy; Consider IV fluid hydration.	None.
≥ 3	Inadequate oral intake; ≥ 6 episodes of vomiting within 24 hours	Maximize anti-emetic therapy; Initiate tube feeding, IVF, or TPN.	If experienced for ≤72 hours, hold MLN0128 (TAK-228) until ≤Grade 1, then resume MLN0128 (TAK-228) without dose modification. If experienced for >72 hours despite optimal therapy, hold MLN0128 (TAK-228) until ≤ Grade 1, then resume treatment with the dose of MLN0128 (TAK-228) reduced by 1 level.
Prevention/Prophylaxis Prophylactic use of anti-emetic, anti-nausea, and anti-diarrheal medications are encouraged and may be used before			

each dose of MLN0128 (TAK-228) as needed throughout the study.
Abbreviations: IV = intravenous; IVF = intravenous fluids; TPN = total parenteral nutrition

6.2.2 Management of Diarrhea

Diarrhea			
Grade	**Description**	**Treatment**	**MLN0128 (TAK-228) Dose Modification**
1	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Loperamide antidiarrheal therapy. Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours)	No change in dose
2	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Same Grade 1	Interrupt MLN0128 (TAK-228) treatment: When symptoms ≤ Grade 1, re-initiate MLN0128 (TAK-228) treatment at a dose reduction Discontinue MLN0128 (TAK-228) treatment if failure to recover within 4 weeks.
3	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL	Same as Grade 1	Interrupt MLN0128 (TAK-228) treatment until symptoms resolve to ≤ Grade 1. Consider re-initiating MLN0128 (TAK-228) treatment at a dose reduction If toxicity recurs at Grade 3, discontinue MLN0128 (TAK-228) treatment.
4	Life-threatening consequences; urgent intervention indicated	Same as Grade 1	Discontinue MLN0128 (TAK-228) treatment.
Abbreviations: ADL = activities of daily living **Prevention/Prophylaxis** Prophylactic use of anti-diarrheal medications are encouraged and may be used before each dose of MLN0128 (TAK-228) as needed throughout the study. a If dose modification is required for subjects receiving ≤ 2 mg QD, then the frequency of dosing should be decreased to QDx5 days/week, rather than decreasing the daily dose administered. Patients requiring > three dose reductions should go off protocol therapy.			

6.2.3 Management of Hyperglycemia

Hyperglycemia			
Grade	**Description**	**Treatment**	**MLN0128 (TAK-228) Dose Modification**
1	Fasting blood sugar > ULN–160 mg/dL	Continue close monitoring of blood sugars. Initiate oral hypoglycemic agent.	None.
2	Fasting blood sugar	Initiate oral hypoglycemic agent and/or insulin if not well controlled on oral agent.	None.

	> 160–250 mg/dL	Endocrinology consult	
3	Fasting blood sugar > 250 -500 mg/dL	Initiate oral hypoglycemic agent and/or insulin, and hydration. Consider inpatient management. Endocrinology consult.	Hold drug until ≤ Grade 2. Resume MLN0128 (TAK-228) based on timing of recovery: ≤ 1 week: resume at same dose and schedule; >1 but ≤ 4 weeks: reduce dose > 4 weeks: stop MLN0128 (TAK-228) and discontinue subject from the study.
4	>500mg/dL	Initiate oral hypoglycemic agent and/or insulin; hydration and inpatient management.	Permanent discontinuation of the study drug
Prevention/Prophylaxis • In-home daily fasting glucose will be monitored. • Follow fasting serum glucose levels during clinic visits. • Life-style modifications, as appropriate (balanced diet, limit alcohol consumption, increase physical activity). • Most episodes of Grade 1 and 2 hyperglycemia respond quickly to oral metformin. • Early initiation of therapy is recommended to prevent higher grade hyperglycemia. • Fasting blood glucose levels ≥ 150 mg/dL by glucometer should be followed by closer monitoring of serum glucose and possible intervention.			
Abbreviations: dL = deciliters; mg = milligrams; ULN = upper limit of normal. a If dose modification is required for subjects receiving ≤ 2 mg QD, then the frequency of dosing should be decreased to QDx5 days/week, rather than decreasing the daily dose administered.			

In the event that any FSG reading performed at the site indicates hyperglycemia (> upper limit of normal [ULN] or ≥ 110 mg/dL), the study staff should first ascertain that the subject was fasting at the time of the blood draw (ie, nothing by mouth for at least 8 hours prior to blood being obtained), had continued to take their concomitant antiglycemic medications should the subject have underlying diabetes mellitus, and repeat the FSG as needed. If the repeat FSG continues to demonstrate hyperglycemia, investigators should initiate steps to aggressively manage the hyperglycemia per standard clinical practice. The following guidelines are provided to aid the investigator in initiating antiglycemic therapies.

Based on the clinical experience from MLN0128 (TAK-228) trials, most episodes of hyperglycemia observed occurred within the first 60 days after initiation of treatment with MLN0128 (TAK-228) and have been either Grade 1 or 2 that have responded quickly to oral metformin. Hyperglycemia has not been dose limiting since instituting a standard regimen for early treatment of hyperglycemia. All subjects developing hyperglycemia on the study should have their glucose closely monitored by study staff. The investigator may choose either to continue close monitoring of subjects who develop Grade 1 hyperglycemia (FSG > ULN ≤ 160 mg/dL) or, alternatively, consider initiating treatment with an oral hypoglycemic agent, such as metformin. All subjects with Grade ≥ 2 hyperglycemia (FSG > 160 mg/dL) must be treated aggressively with oral hypoglycemic agents and/or insulin as clinically indicated while continuing on MLN0128 (TAK-228). The investigator should consult an endocrinologist if needed to aid in optimizing the subject's hyperglycemia treatment plan.

It is recommended that subjects be treated initially with a fast acting, insulin sensitizer, such as metformin at 500 mg PO QD, and titrate up to a maximum of 1000 mg PO BID as needed. Concurrent addition to metformin of DPP-4 inhibitors (eg, sitagliptin or vildagliptin) and/or insulin should also be considered. Oral sulfonylureas (eg, glipizide or glyburide) should be used with caution due to the higher risk of inducing hypoglycemia in subjects. The dose of oral hypoglycemic agents should be adjusted in subjects with renal insufficiency. In addition, patients should be encouraged to follow a low carbohydrate diet once hyperglycemia is first observed.
If any fasting serum glucose reading performed at the site indicates hyperglycemia (>ULN or ≥110 mg/dL), the study staff should first confirm that the patient was fasting at the time of blood specimen collection (ie, nothing by mouth for at least 8 hours before collection).

In-Home Daily Fasting Glucose Monitoring
In addition to obtaining fasting glucose levels at the clinic visits as outlined in the Schedule of Events, all patients randomized to receive MLN0128 (TAK-228) will be given a glucometer to monitor their daily FBG levels at home. The level should be collected daily, predose on dosing days, and at approximately the same time each day.

On Cycle 1 Day 1, the patient will be provided an in-home glucometer. Patients will be trained on proper use of the glucometer and instructed to collect a daily FBG level every morning (predose on dosing days), starting on Cycle 1 Day 2. Patients will be instructed to bring the glucometer with them to each study visit so that the data collected can be reviewed and recorded in the source documents. Investigators will be responsible for reviewing the home glucose monitoring logs for hyperglycemia.

The patient will be instructed to contact the site immediately if the value is abnormal (ie, ≥150 mg/dL) for further instructions on the management of their hyperglycemia. Hyperglycemia observed during home glucose monitoring should be confirmed in the clinic.

If no irregularities in the fasting blood glucose level are observed during a minimum of 2 consecutive months, then the frequency of in-home fasting blood glucose testing can be reduced to a minimum frequency of once weekly, depending on the investigator's judgment and approval. Patients will continue to notify the investigator of fasting blood glucose levels that exceed 150 mg/dL and, if blood glucose levels are not well controlled, or if the patient requires either oral hypoglycemic agents or insulin to control blood glucose levels, then the frequency of in-home testing of FBG levels will be reinstated to daily.

6.2.4 Management of Non-infectious Pneumonitis

Non-infectious Pneumonitis			
Grade	**Description**	**Treatment**	**MLN0128 (TAK-228) Dose Modification**
1	Asymptomatic: Radiographic findings only	Rule out infection and closely monitor.	None.
2	Symptomatic:	Rule out infection and consider treatment with	Interrupt MLN0128 (TAK-228) treatment:

Non-infectious Pneumonitis			
Grade	**Description**	**Treatment**	**MLN0128 (TAK-228) Dose Modification**
	Not interfering with ADLs	corticosteroids until symptoms improve to ≤ Grade 1.	When symptoms ≤ Grade 1, re-initiate MLN0128 (TAK-228) treatment at a dose reduction Discontinue MLN0128 (TAK-228) treatment if failure to recover within 4 weeks.
3	Symptomatic: Interfering with ADLs; Requires administration of O_2	Rule out infection and consider treatment with corticosteroids until symptoms improve to ≤ Grade 1.	Interrupt MLN0128 (TAK-228) treatment until symptoms resolve to ≤ Grade 1. Consider re-initiating MLN0128 (TAK-228) treatment at a dose reduction If toxicity recurs at Grade 3, discontinue MLN0128 (TAK-228) treatment.
4	Life-threatening: Ventilatory support indicated	Rule out infection and consider treatment with corticosteroids.	Discontinue MLN0128 (TAK-228) treatment.
Abbreviations: ADL = activities of daily living; O_2 = oxygen gas. a If dose modification is required for subjects receiving ≤ 2 mg QD, then the frequency of dosing should be decreased to QDx5 days/week, rather than decreasing the daily dose administered.			

6.2.5 Management of Hyperlipidemia

Hyperlipidemia		**Management/Next Dose for MLN0128 (TAK-228)**
Grade 1	Cholesterol: > ULN - 300 mg/dL Triglycerides: > 150 - 300 mg/dL	No change in dose
Grade 2	Cholesterol: > 300 – 400 mg/dL Triglycerides: > 300 - 500 mg/dL	Treat hyperlipidemia according to standard guidelines. Triglycerides ≥ 500 mg/dl should be treated urgently due to risk of pancreatitis. Maintain dose if tolerable. If toxicity becomes intolerable, interrupt MLN0128 (TAK-228) dosing until recovery to ≤ Grade 1. Reinitiate at same dose.
Grade 3	Cholesterol: > 400 - 500 mg/dL Triglycerides: > 500 - 1000 mg/dL	Treat hyperlipidemia according to standard guidelines. Triglycerides ≥ 500 mg/dl should be treated urgently due to risk of pancreatitis. Hold dose until recovery to ≤ Grade 1, then restart at a dose reduction.
Grade 4	Cholesterol: > 500 mg/dL Triglycerides: > 1000 mg/dL	Treat hyperlipidemia according to standard guidelines. Triglycerides ≥ 500 mg/dl should be treated urgently due to risk of pancreatitis. Discontinue treatment.
Prevention/Prophylaxis		

Hyperlipidemia	Management/Next Dose for MLN0128 (TAK-228)
• Life-style modifications, as appropriate (balanced diet, limit consumption of alcoholic beverages, increase physical activity).	
Abbreviations: dL = deciliters; mg = milligrams; ULN = upper limit of normal. a If dose modification is required for subjects receiving ≤ 2 mg QD, then the frequency of dosing should be decreased to QDx5 days/week, rather than decreasing the daily dose administered.	

6.2.6 Management of Oral Mucositis

Oral Mucositis			
Grade	**Description**	**Treatment**	**MLN0128 (TAK-228) Dose Modification**
1	Asymptomatic or mild symptoms	Non-alcoholic mouth wash or 0.9% salt water rinse; Consider topical corticosteroids at earliest signs of mucositis.	None.
2	Moderate pain, not interfering with oral intake Modified diet indicated	Topical analgesic mouth treatments; Topical corticosteroids; Initiate antiviral or antifungal therapy, if indicated.	Maintain dose if tolerable. If toxicity becomes intolerable, interrupt MLN0128 (TAK-228) dosing until recovery to ≤ Grade 1. Reinitiate at same dose.
3	Severe pain, interfering with oral intake	Same as for Grade 2; Consider intra-lesional corticosteroids.	Hold dose until recovery to ≤ Grade 1, then restart at a dose reduction
4	Life-threatening consequences	Same as for Grade 2. Consider intra-lesional corticosteroids.	Discontinue treatment.
Prevention/Prophylaxis • Consider initiation of a non- alcoholic mouth wash or 0.9% salt water rinses 4-6 times daily with start of therapy before signs of mucositis develop. • Avoid using agents containing hydrogen peroxide, iodine, and thyme derivatives in management of stomatitis as they may worsen mouth ulcers.			
a If dose modification is required for subjects receiving ≤ 2 mg QD, then the frequency of dosing should be decreased to QDx5 days/week, rather than decreasing the daily dose administered.			

6.2.7 Management of Rash

Rash			
Grade	**Description**	**Treatment**	**MLN0128 (TAK-228) Dose Modification**
≤ 2	Macules/papules covering ≤ 30% body surface area with or without symptoms	Consider treatment with topical steroid cream/ointment and/or oral anti-histamines.	None.
3	Macules/papules covering > 30% body surface area with or without symptoms	Consider treatment with topical steroid cream/ointment, oral antihistamines, and/or pulsed steroids. Consult dermatology.	Hold until ≤ Grade 2; Resume MLN0128 (TAK-228) based on timing of recovery: ≤ 4 weeks: reduce dose ; > 4 weeks: stop MLN0128 (TAK-228) and discontinue subject from the study.

4	acneifom/papulopustular rash with papules and/or pustules covering any % body surface area, which may or may not be associated with symptoms of pruritus or tenderness, and **are associated with extensive superinfection with intravenous (IV) antibiotics indicated;** life threatening consequences.	All the above; IV antibiotics for superinfection. Consult dermatology.	Permanent discontinuation of the study drug.
a If dose modification is required for subjects receiving ≤ 2 mg QD, then the frequency of dosing should be decreased to QDx5 days/week, rather than decreasing the daily dose administered.			

Patients who develop Grade 4 rash should permanently discontinue study treatment. Grade 4 rash is defined as rash acneifom/papulopustular with papules and/or pustules covering any % body surface area, which may or may not be associated with symptoms of pruritus or tenderness, and are associated with extensive superinfection with intravenous (IV) antibiotics indicated; life threatening consequences (NCI CTCAE Version 5.0, effective date April 1, 2018).

6.2.8 Management of QTc Prolongation

QTc Prolongation			
Grade	**Description**	**Treatment**	**MLN0128 (TAK-228) Dose Modification**
2	480 ms < QTc < 501 ms	Evaluate for other possible causes (eg, electrolyte disturbance, concomitant medication, etc.)	None; continue MLN0128 (TAK-228) at the same dose and schedule.
≥ 3	QTc ≥ 501 ms	Evaluate for other possible causes (eg, electrolyte disturbance, concomitant medication)[a]; Consider a formal consult by a cardiologist; Notify the study doctor; Additional ECGs may be performed at intervals that the treating physician deems clinically appropriate until repeated QTc measurements fall or are below the threshold interval that triggered the repeat measurement.	MLN0128 (TAK-228) should be interrupted. Patients who experience persistent symptomatic Grade 3 or Grade 4 QTc prolongation without another cause should permanently discontinue study treatment.
Abbreviations: ECG = electrocardiogram; IV = intravenous; ms = milliseconds; QTc = QT interval corrected for heart rate A list of medications known to prolong QTc can be found at www.torsades.org and www.QTdrugs.org.			

6.2.9 Management of elevated creatinine

Elevations in creatinine	Management/Next Dose for MLN0128 (TAK-228)
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Off protocol therapy
*Patients requiring a delay of >4 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	
Further evaluation of the renal insufficiency should be performed to rule out other reversible causes like hypovolemia or hydronephrosis. Subjects should be encouraged to drink at least 18-24 ounces of fluids a day, especially on days	

Elevations in creatinine	Management/Next Dose for MLN0128 (TAK-228)
requiring fasting (as per protocol), with administration of IV fluids in the clinic as clinically indicated to avoid dehydration. Microscopic urinalysis, a 12-hour urine collection, spot urine electrolytes, protein and creatinine, and serum chemistry should be collected at any time when the serum creatinine is ≥ Grade 1.	

6.2.10 Management of Transaminitis

Transaminitis	Management/Next Dose for MLN0128 (TAK-228)
≤ Grade 1	No change in dose
Grade 2	Hold * until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Off protocol therapy
*Patients requiring a delay of >4 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting (via CTEP-AERS) **in addition** to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks List(s) (CAEPRs)

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
MLN0128 (TAK-228, NSC 768435)

The Comprehensive Adverse Events 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 390 patients.* Below is the CAEPR for MLN0128 (TAK-228).

> **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.

7.1.1 CAEPRs for CTEP IND Agent, MLN0128 (TAK-228)

Version 2.3, July 28, 2019[1]

Adverse Events with Possible Relationship to MLN0128 (TAK-228) (CTCAE 5.0 Term) [n= 390]			**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)***
	Dyspepsia		
Mucositis oral			***Mucositis oral (Gr 2)***
Nausea			***Nausea (Gr 3)***
Vomiting			***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		
Fatigue			***Fatigue (Gr 3)***
	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			
	Urinary tract infection		***Urinary tract infection (Gr 2)***
INVESTIGATIONS			
	Creatinine increased		***Creatinine increased (Gr 2)***
		Electrocardiogram QT corrected interval prolonged	
	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 3)***
	Hypokalemia		***Hypokalemia (Gr 2)***
	Hypomagnesemia		***Hypomagnesemia (Gr 2)***
	Hypophosphatemia		***Hypophosphatemia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
	Back pain		***Back pain (Gr 2)***
	Pain in extremity		
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Dysgeusia		***Dysgeusia (Gr 2)***
	Headache		***Headache (Gr 2)***
PSYCHIATRIC DISORDERS			
	Insomnia		

Adverse Events with Possible Relationship to MLN0128 (TAK-228) (CTCAE 5.0 Term) [n= 390]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
RENAL AND URINARY DISORDERS			
	Acute kidney injury		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 2)***
	Oropharyngeal pain		
		Pneumonitis	
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.

Adverse events reported on MLN0128 (TAK-228) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that MLN0128 (TAK-228) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (hyperviscosity syndrome); Blood and lymphatic system disorders - Other (Raynaud's phenomenon); Febrile neutropenia
CARDIAC DISORDERS - Heart failure; Pericardial effusion; Sinus tachycardia; Ventricular arrhythmia
EYE DISORDERS - Blurred vision; Eye pain; Photophobia; Vision decreased
GASTROINTESTINAL DISORDERS - Abdominal distension; Colitis; Dysphagia; Esophagitis; Gastritis; Gastroesophageal reflux disease; Gastrointestinal disorders - Other (intestinal obstruction); Gastrointestinal disorders - Other (intestinal perforation); Gastrointestinal disorders - Other (salivary hypersecretion); Hemorrhoids; Ileus; Oral pain; Pancreatitis; Small intestinal obstruction; Small intestinal perforation; Toothache
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Flu like symptoms; Gait disturbance; General disorders and administration site conditions - Other (groin pain); Malaise; Non-cardiac chest pain; Pain
HEPATOBILIARY DISORDERS - Gallbladder obstruction
IMMUNE SYSTEM DISORDERS - Allergic reaction
INFECTIONS AND INFESTATIONS - Abdominal infection; Infections and infestations - Other (cystitis); Infections and infestations - Other (lower respiratory tract infection); Infections and infestations - Other (mucosal infection); Infections and infestations - Other (parotid gland); Kidney infection; Lung infection; Papulopustular rash; Sepsis; Skin infection; Upper respiratory infection
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fall; Fracture; Injury, poisoning and procedural complications - Other (accidental overdose); Injury, poisoning and procedural complications - Other (postoperative fever); Injury, poisoning and procedural complications - Other (subdural hemorrhage); Tracheal obstruction
INVESTIGATIONS - Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Blood lactate dehydrogenase increased; Cholesterol high; GGT increased; Lipase increased; Lymphocyte count decreased; Neutrophil count decreased; White blood cell decreased

METABOLISM AND NUTRITION DISORDERS - Acidosis; Hypercalcemia; Hyperkalemia; Hypernatremia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hyponatremia; Metabolism and nutrition disorders - Other (severe chronic malnutrition); Metabolism and nutrition disorders - Other (vitamin D deficiency)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Chest wall pain; Flank pain; Generalized muscle weakness; Muscle cramp; Myalgia
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (non-hodgkin lymphoma); Treatment related secondary malignancy
NERVOUS SYSTEM DISORDERS - Ataxia; Intracranial hemorrhage; Lethargy; Nervous system disorders - Other (carotid artery occlusion); Nervous system disorders - Other (neuropathy peripheral); Paresthesia; Radiculitis; Stroke; Tremor
PSYCHIATRIC DISORDERS - Anxiety; Confusion; Depression; Psychiatric disorders - Other (mental status changes)
RENAL AND URINARY DISORDERS - Dysuria; Hematuria; Proteinuria; Renal and urinary disorders - Other (strangury)
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Vaginal hemorrhage
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchopulmonary hemorrhage; Epistaxis; Hiccups; Hypoxia; Nasal congestion; Pleural effusion; Pleuritic pain; Pneumothorax; Postnasal drip; Productive cough
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Rash acneiform; Urticaria
VASCULAR DISORDERS - Flushing; Hypertension; Hypotension; Thromboembolic event

Note: MLN0128 (TAK-228) 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 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 until March 31, 2018 for AE reporting. CTCAE version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 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, Section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs for the protocol that do not require expedited reporting are outlined in section 7.3.4.

- **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.

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (https://eapps-ctep.nci.nih.gov/ctepaers). 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/protocolDevelopment/electronic_applications/adverse_events.htm). 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 Distribution of Adverse Event Reports

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and Adverse Event Coordinator(s) (if applicable) of the Corresponding Organization or Lead Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

The Coordinating Center of the Corresponding Organization is responsible for submitting to the CTSU documentation of AEs that they deem reportable for posting on the CTSU protocol web page and inclusion on the CTSU bi-monthly broadcast.

7.3.3 Expedited Reporting Guidelines

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. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions." 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.

The Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse

Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of MLN0128 (TAK-228)[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 AdEERS within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">10 Calendar Days</td><td rowspan="2">24-Hour 5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not required</td><td>10 Calendar Days</td></tr>
</table>

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 AdEERS 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 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

[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

7.4 Routine Adverse Event Reporting

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

Serious AE (SAE) means any untoward medical occurrence that at any dose:

- Results in **death.**
- Is **life-threatening** (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient **hospitalization or prolongation of an existing hospitalization** (see clarification in the paragraph below on planned hospitalizations).
- Results in **persistent or significant disability or incapacity**. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- Is a **congenital anomaly/birth defect**.
- Is a **medically important event**. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent 1 of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse; any organism, virus, or infectious particle (eg, prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent.

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term *severe* is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as *serious*, which is based on patient/event outcome or action criteria described above, and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm^3 to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

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 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

7.7 Pregnancy

Although not an adverse event in and of itself, pregnancy as well as its outcome must be documented via **CTEP-AERS**. In addition, the ***Pregnancy Information Form*** included within the NCI Guidelines for Adverse Event Reporting Requirements must be completed and submitted to CTEP. Any pregnancy occurring in a patient or patient's partner from the time of consent to 90 days after the last dose of study drug must be reported and then followed for outcome. Newborn infants should be followed until 30 days old. Please see the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" (at http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm) for more details on how to report pregnancy and its outcome to CTEP.

7.8 Pregnancy loss

- Pregnancy loss is defined in CTCAE as "Death in utero."
- Any Pregnancy loss should be reported **expeditiously**, as Grade 4 "Pregnancy loss" under the Pregnancy, puerperium and perinatal conditions SOC.
- A Pregnancy loss should NOT be reported as a Grade 5 event under the Pregnancy, puerperium and perinatal conditions SOC, as currently CTEPAERS recognizes this event as a patient death.

7.9 Neonatal death

A neonatal death should be reported **expeditiously** as Grade 4, "Death neonatal" under the General disorders and administration SOC.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the use of MLN0128 (TAK-228) can be found in Section 7.1.

8.1 CTEP IND Agent(s)

8.1.1 *MLN0128 (TAK-228)*

Other Names: TAK-228, INK128

Other Names: TAK-228, 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 (mechanistic 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 is supplied by Millennium Pharmaceuticals, Inc. and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI as size 2 hard gelatin capsules in the following strengths: 1 mg (white opaque color), and 3 mg (orange opaque color). The composition of the drug product consists of a blend of MLN0128 drug substance, microcrystalline cellulose, and magnesium stearate. **Milled** formulations will have a white label with a large watermark of the strength on the label.

MLN0128 capsules are packaged in 30-count, 60-cc high-density polyethylene (HDPE), white, opaque, round, tamper- and child-resistant bottles.

Storage: Capsules are to be stored in the original package between 15°C to 30°C, with allowed short-term excursions between 2°C and 40°C.

Route of Administration: MLN0128 (TAK-228) should be taken orally on an empty stomach at least 2 hours after food and do not eat or drink (except water) for at least one hour after taking MLN0128 (TAK-228). Do not chew, open or manipulate the capsule in any way prior to swallowing. Each dose should be taken with 8 ounces (240 mL) of water.

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.

Patient Care Implications:
Women of childbearing potential should use effective methods of contraception during and through 90 days after the last dose of MLN0128.

Men should use effective methods of contraception and not donate sperm during and through 120 days after the last dose of MLN0128.

8.1.2 Agent Ordering and Agent Accountability

8.1.2.1 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 (301) 496-5725 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

8.1.2.2 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 Oral Drug Accountability Record Form (DARF). (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.2.3 Study drug will be administered or dispensed only to eligible patients under the supervision of the investigator or identified sub-investigator(s). The appropriate study personnel will maintain records of study drug receipt and dispensing.

8.1.2.4 IB documents for agents on protocols where CTEP/DCTD sponsors the IND and PMB distributes the agent are available via PMB's Online Agent Order Processing (OAOP) application. Access to OAOP requires an active CTEP IAM account with a current password. Once logged into OAOP, go to the "Investigator Brochures" tab and provide the required investigator, agent, and protocol information to search for the current IB documents. Refer to PMB's FAQs for more information about accessing OAOP and obtaining an investigator brochure.

8.1.2.5 Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: PMBRegPend@ctep.nci.nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx
- CTEP Identity and Access Management (IAM) account: https://eapps-ctep.nci.nih.gov/iam/
- CTEP Associate Registration and IAM account help: ctepreghelp@ctep.nci.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov
  PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

Hypotheses: MLN0128 (TAK-228) is active, safe and tolerable in patients with locally advanced or metastatic TCC harboring a *TSC1* mutation

This study will be using an integral biomarker by identifying bladder cancer patients whose tumors harbor a TSC1 and/or TSC2 mutation identified by a CLIA-certified laboratory. If the patient's mutation status is unknown, this protocol screens patients' TCC tumor for *TSC-1 a*nd/or *TSC-2* mutation at the Yale Clinical Molecular Pathology Laboratory (YCMPL) under the direction of Dr. Jeffrey Sklar. Only patients whose TCC tumors are determined to be positive for TSC1 and/or TSC2 mutations will be treated with MLN0128 (TAK-228). Unless the prescreening was done at YCMPL, the participating site must submit an archival tissue prior to initiation of treatment in a form of 10 unstained slides, or a FFPE block to Dr. Jeffrey Sklar's laboratory at Yale University. Contact the Study PI via email for the latest version of the Lab Manual for handling the specimen. This will be posted on the CTSU website.

Background: As previously noted, only those patients whose tumors harbor mutations in the

TSC1 and/or *TSC2* and meet other eligibility criteria will be treated with MLN0128 (TAK-228). It is unknown if the biologic and clinical effects of *TSC1* and *TSC2* mutations in TCC are identical. The available literature supports a positive correlation between *TSC1* mutation and clinical benefit with rapalog treatment; however, it is unknown if the same benefit is possible with *TSC2* mutated TCC. The primary objective will be to study the activity of MLN0128 (TAK-228) in *TSC1* mutated TCC. It is unknown if the biologic and clinical effects of *TSC1* and *TSC2* mutations are identical. We will evaluate the activity and tolerability of MLN0128 (TAK-228) in *TSC2* mutated TCC in an exploratory fashion to gain experience as we accrue in the TSC1 cohort to meet its accrual objectives of evaluable patients.

Facilities and methods: Dr. Jeffrey Sklar at Yale University will implement a clinically validated program for assessing somatic coding mutations in the *TSC1* and *TSC2* genes in bladder cancers. The profiling will be done at the YCMPL which operates under the auspices of CMS/CLIA. Paraffin embedded tissue blocks or slides will be provided by the clinical sites and shipped to Dr. Sklar's laboratory using a standard operating procedure (SOP) developed and validated and currently being used for the NCI MATCH program. Specimens will be histopathologically assessed and DNA will be subsequently extracted from each quality assessed tumor using Yale's Molecular Profiling SOPs.

The above mentioned samples will be assessed for adequacy and DNA will be extracted. *TSC1* and *TSC2* genes will be sequenced using a validated CLIA certified approach. To address reportable range, nucleotide variants of TSC1 and TSC2 will be counted in treatment decisions only if they meet all of the following criteria: 1) the specific nucleotide change is not found or at prevalence below 0.001 percent within the 1000 Genomes database; 2) the specific nucleotide change is not found within the NHLBI exome sequence variant database; 3) The specific nucleotide variant resides within an annotated coding exon of TSC1 and/or TSC2; 4) The specific nucleotide variant results in an amino acid change, stop codon, or a frameshift.

9.1.1 **Tissue Handling and Shipping:**

Contact the Study PI for the Lab Manual. This will be posted on the CTSU website.

10. STUDY CALENDAR

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.

For each of visits, there is +/- 3 business day window in case of scheduling conflicts.

			Cycle 1		Cycle 2		Cycle 3 and Subsequent Cycles	
Assessments[1]	Pre-Screen[9]	Screening Day -28 to Day -1	Cycle 1 Day 1	Cycle 1 Day 15	Cycle 2 Day 1	Cycle 2 Day 15	Day 1	End of Treatment[15]
Pre-Screening Consent	X							
Archival Tissue submission for TSC1 or TSC2 mutation testing	X							
Buccal swab	X							
Study Consent		X						
Registration		X[13]						
Concurrent meds		X	X	X	X	X	X	X
Performance Status check		X	X	X	X	X	X	X
Physical Exam[2]		X	X	X	X	X	X	X
Vitals		X	X	X	X	X	X	X
Hematology/ Chemistry[3]		X	X	X	X	X	X	X
Coagulation (PT/INR, aPTT)		X	X		X		X	X
Fasting serum glucose[6]		X	X	X	X	X	X	X
Fasting lipid profile[4]		X	X		X		X	X
Pregnancy test[10]		X[10]	X[10]		X[10]		X[10]	X[10]
Urinalysis		X	X	X	X	X	X	X
EKG[5]		X						X
Archival tumor tissue [14] (or fresh tumor biopsy[12a]), and buccal swab		X						X[12b]
MLN0128 (TAK-228) Admin			X----------→					
AE assessment			X	X	X	X	X	X
Tumor measurements[7] (CT, MRI[8])		X	Tumor measurements are repeated after every 8 weeks for the first 24 weeks, then after every 12 weeks thereafter. Confirmatory scans will also be obtained 4 to 8 weeks following initial documentation of an objective response					
Completed Diary[11] Returned to Clinic				X	X	X	X	X

1. Assessments (safety labs including chemistry/hematology/coag, fasting lipid profiling, pregnancy test, urinalysis) may be done within 48 hours of dosing with MLN0128 (TAK-228) administration.
2. Symptom directed.
3. CBC with differential, electrolyte panel, BUN, Serum Creatinine, LFTs (AST, ALT, Alk Phos, Total and Direct bilirubin), Albumin, Calcium, Phosphate, Magnesium, LDH, total protein.
4. Total cholesterol, HDL-C, LDL-C, triglycerides.
5. Single, 12-lead EKG will be collected as screening (within 28 of first dose) at the end of treatment and on therapy if clinically indicated.
6. Fasting serum glucose will be measured in the clinic. Patients are required to fast overnight (nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment) for each of these measurements.
7. Tumor measurements will follow RECIST v1.1. Standard of Care modalities will be utilized to collect tumor measurements for study subjects. The same modality used at screening will be followed throughout the study period.
8. Brain MRI or CT head with IV contrast (if contraindication to MRI) should be considered in a patient with history of CNS metastasis or when clinically indicated.
9. Consent for Prescreening testing and the tissue submission can be done at any time in the subject's metastatic disease status as long as the patient is potentially deemed eligible for the Main Study in the opinion of the treating investigator.
10. Pregnancy Testing is required for women of childbearing potential. While serum beta-HCG testing is required for screening, either serum or urine pregnancy test is required prior to the start of each cycle beginning cycle 1, at End of Treatment and End of Study. In a scenario where a beta-HCG elevation is noted as a serum tumor marker of TCC, a series of beta HCG levels must be documented to prove that the level and the trend of beta-HCG elevation are not consistent with pregnancy. A pelvic/pregnancy US should be considered if pregnancy is suspected.

11. Please return any diaries and pill bottles to each study visit, beginning with C1D15. Pill diary include **DAILY glucose monitoring. Glucometer will be provided to the patient.**
12a. A tumor biopsy is mandatory for those patients who have no available tissue for submission.
12b. A tumor biopsy is optional at the time of disease progression; recommended in patients with initial response to TAK-228.
13. Patients should begin protocol treatment within 7 days following registration. Issues that would cause treatment delays should be discussed with the Principal Investigator
14. Archival tumor tissue submission is required **unless** the pre-screening was done at YCMPL. (i.e., if pre-screening for *TSC* mutation was done at YCMPL, then no additional tissue submission is needed). If no archival tumor tissue available, a fresh tumor biopsy is required. (See criteria 3.1.4)
15. EOT visit will take place at 4 weeks after the last dose of the study drug (See the Section 5.4)

11. MEASUREMENT OF EFFECT

Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response after every 8 weeks for the first 24 weeks, then after every 12 weeks thereafter. In addition to a baseline scan, confirmatory scans should also be obtained in 4 to 8 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) [*Eur J Ca* 45:228-247, 2009]. 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 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with MLN0128 (TAK-228)

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.

11.2 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.

11.3 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 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. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity. mTOR inhibitors inhibit GLUT1 and other cellular glucose transporters, and MLN0128 (TAK-228) might potentially decrease FDG uptake in a residual mass as a pharmacokinetic effect separate from an antitumor effect. Therefore, FDG PET scans taken concurrent with MLN0128 (TAK-228) treatment may potentially give a false negative reading.

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.

11.4 Response Criteria

11.4.1 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.

11.4.2 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).

11.4.3 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

11.4.4 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.

11.4.5 Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of treatment to date of progression or death, whichever occurs first. Patients not known to have progressed will be censored for PFS as of the date of their last tumor assessment performed via the methods described in Section 11.1.

11.4.6 Overall Survival

Overall survival (OS) is defined as the duration of time from start of treatment to time of death from any cause. Patients not known to have expired will be censored for OS as of the most recent date of verification of their being still alive.

12. STUDY OVERSIGHT AND 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 Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through the CTMS web-based reporting portal.

For this Phase 2 study, the Protocol Principal Investigator will have, at a minimum, quarterly conference calls with the Study Investigators and the CTEP Medical Officer(s) to review accrual,

progress, and pharmacovigilance. Decisions to proceed to the second stage of a Phase 2 trial will require sign-off by the Protocol Principal Investigator and the Protocol Statistician.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via Medidata Rave.

All studies are also reviewed in accordance with the enrolling institution's data safety monitoring plan.

12.2 Data Reporting

Data collection for this study will be done exclusively through Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in the Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP IAM account (check at < https://ctepcore.nci.nih.gov/iam >) and the appropriate Rave role (Rave CRA, Read-Only, CRA, Lab Admin, SLA or Site Investigator) on either the LPO or participating organization roster at the enrolling site. To the hold Rave CRA role or CRA Lab Admin role, the user must hold a minimum of an AP registration type. To hold the Rave Site Investigator role, the individual must be registered as an NPIVR or IVR. Associates can hold read-only roles in Rave. If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

12.2.1 Method

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at: http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. On-

site audits will be conducted on an 18-36 month basis as part of routine cancer center site visits. More frequent audits may be conducted if warranted by accrual or due to concerns regarding data quality or timely submission. For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 799-7580 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs

12.2.2 Responsibility for Data Submission

For ETCTN trials, it is the responsibility of the PI(s) at the site to ensure that all investigators at the ETCTN Sites understand the procedures for data submission for each ETCTN protocol and that protocol specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system, Medidata Rave.

Data are to be submitted via Medidata Rave to CTMS on a real-time basis, but no less than once every 2 weeks. The timeliness of data submissions and timeliness in resolving data queries will be tracked by CTMS. Metrics for timeliness will be followed and assessed on a quarterly basis. For the purpose of Institutional Performance Monitoring, data will be considered delinquent if it is greater than 4 weeks past due.

Data from Medidata Rave and CTEP-AERS is reviewed by the CTMS on an ongoing basis as data is received. Queries will be issued by CTMS directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the ETCTN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted by the CTMS to ensure compliance with regulatory requirements, GCP, and NCI policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, as described in the ETCTN Program Guidelines, which may be found on the CTEP (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm) and CTSU websites.

An End of Study CRF is to be completed by the PI, and is to include a summary of study endpoints not otherwise captured in the database, such as (for phase 1 trials) the recommended phase 2 dose (RP2D), and a description of any dose-limiting toxicities (DLTs). CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

CDUS data submissions for ETCTN trials activated after March 1, 2014, will be carried out by the CTMS contractor, Theradex. CDUS submissions are performed by Theradex on a monthly basis. The trial's lead institution is responsible for timely submission to

CTMS via Rave, as above.

Further information on data submission procedures can be found in the ETCTN Program Guidelines (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm).

12.3 CTEP Multicenter Guidelines

12.4 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are 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

The study **design** is a single arm 2-stage Phase II pilot study of MLN0128 (TAK-228) in bladder

cancer patients whose tumors harbor a *TSC1* mutation.

As per Section 1.1, the primary objective is to estimate the overall response rate (ORR) observed among TSC1 patients. Hence, the primary statistical **endpoint** is the ORR among TSC1 patients.

As per Section 1.2, the secondary **endpoints** are:

a) Toxicity among TSC1 patients;
b) PFS and OS among TSC1 patients.

As per Section 1.3, the exploratory **endpoints** are:

a) ORR among TSC2 patients;
b) Toxicity among TSC2 patients;
c) PFS and OS among TSC2 patients.

13.2 Sample Size/Accrual Rate

13.2.1 To minimize the total number of patients with TSC1 mutant urothelial carcinomas (TSC1 patients) potentially needed for the planned 1-sample, 2-stage Phase 2 trial, a Simon minimax study design will be used. We have assumed an ORR 0.10 (10%) for current therapy. We believe that an improvement to 0.30 (30%) with MLN0128 (TAK-228) treatment would be clinically meaningful. We have also assumed alpha = 0.10 and power = 0.90. Under those assumptions a maximum of 25 eligible TSC1 patients would be required, 16 in Stage 1 and an additional 9 in Stage 2 (if needed), as determined by PASS 12 software.

Assuming an approximate rate of 12% TSC1 incidence, it is estimated that 134 patients would need to be enrolled and screened in order to obtain the Stage 1 statistical design requirement of 16 eligible TSC1 patients.

If Stage 2 were required, it is estimated that 75 additional patients would need to be enrolled and screened in order to find 9 more TSC1 patients. That would mean screening a total of 209 patients to find the maximum statistical design requirement of 25 eligible TSC1 patients.

13.2.2 Stopping Rules
In Stage 1, 16 eligible TSC1 patients will be accrued. A minimum follow-up period of 12 weeks will be required to assess clinical response. As per 11.4.3, all the responses will be confirmed with a subsequent scan obtained $\geq$ 4 weeks. If $\leq$ 1 response is observed among them, stop the study and conclude that MLN0128 (TAK-228) is not worthy of further evaluation in this patient population. If $\geq$ 2 responses are observed, accrue an additional 9 eligible TSC1 patients (i.e., begin Stage 2 of patient accrual). If $\leq$ 4 responses are observed in the final set of 25 eligible TSC1 patients, conclude that MLN0128 (TAK-228) is not worthy of further study in TSC1 patients. If $\geq$ 5 responses are observed, conclude that MLN0128 (TAK-228) is promising.

The Type I error (i.e., the probability of concluding that MLN0128 (TAK-228) is promising when

that is not true) is 0.095 if the true response rate is 10%. The power (i.e., the probability of correctly concluding that the regimen is active) of this procedure is 0.903 when the true response rate is 30%. The probability of early termination (PET) with this design is 0.515 when the true response rate is 10%, and the average total sample size for this design is 20.1 eligible TSC1 patients.

13.2.3 These assumptions have been made regarding TSC1 (and TSC2) patient accrual rate.

TSC1: Metastatic bladder cancer patients will be recruited through the ETCTN. Accessibility of these patients will come from both the UM-1 and N01 holders. We do not anticipate a recruitment deficiency. Additionally, there were 4 lead institutions initially involved in the design and development of this protocol. Those institutions are still interested and are: Karmanos, Dana Farber Cancer Institute, Vanderbilt and Yale. These 4 institutions see approximately 14, 150, 30 and 60 metastatic bladder cancer patients per year respectively, for a total of 254 such patients/year. Conservatively, we expect that about 15% of them will have a TSC1 (12%) and/or TSC2 (3%) gene mutation, making them study eligible. That 12% TSC1 incidence yields an estimated combined accrual rate of about 30 eligible ***TSC1*** patients/year.

Allowing for up to 10% attrition of patients who do not become eligible, we may have to enroll 28 eligible TSC1 patients in order to have the potential maximum of 25 who are eligible for our planned 2-stage Simon minimax design. At the expected accrual rate of 30 eligible TSC1 patients/year, it would take ~12 months to accrue 25 eligible TSC1 patients for Stage 2 (should Stage 2 be needed).

TSC2: Since TSC2 patients only appear in our Exploratory Objectives, their accrual will be halted whenever the appropriate number of eligible TSC1 patients have been accrued, as per the planned 2-stage Simon minimax design.

Hence, it is uncertain how many TSC2 patients will be accrued, although the 3% incidence rate mentioned above might be a guideline. That is about ¼ of the expected incidence rate of TSC1 patients. So, if 25 eligible TSC1 patients are eventually enrolled, then about 6-7 TSC2 patients would be expected to have been enrolled in parallel.

If any patients are enrolled who have *both* TSC1 *and* TSC2 mutations, they will be included with the exploratory cohort of patients who have *only* TSC2 mutations. In this way, all interpretations of study results for the Primary and Secondary Objectives can be restricted to patients who have o*nly* a TSC1 mutation.

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	2	+	7	=	9
Not Hispanic or Latino	3	+	13	=	16
Ethnic Category: Total of all subjects	5	+	20	=	25 (C1)

Racial Category					
American Indian or Alaskan Native	1	+	3	=	4
Asian	1	+	3	=	4
Black or African American	1	+	4	=	5
Native Hawaiian or other Pacific Islander	0	+	3	=	3
White	2	+	7	=	9
Racial Category: Total of all subjects	5 (A2)	+	20 (B2)	=	25 (C2)
	(A1 = A2)		(B1 = B2)		(C1 = C2)

13.3 Stratification Factors

Not applicable since this is a 1-arm study. Any stratification of interest can be performed after accrual is completed and the data are collected.

13.4 Analysis of Secondary Endpoints

As per Section 1.2, the secondary objectives are:

1) To evaluate the safety and tolerability of MLN0128 (TAK-228) in patients with locally advanced or metastatic TCC harboring a *TSC1* mutation.

2) To evaluate progression free survival (PFS) and overall survival (OS).

For Secondary Objective 1, the occurrence rate of each specific type of toxicity at a certain severity grade will be described by point estimates and Wilson type 90% (2-sided) confidence intervals (CIs).

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For Secondary Objective 2, the censored PFS and OS distributions will each be estimated by the Kaplan-Meier (K-M) survivorship function. Point estimates and 90% (2-sided) CIs will be computed for all estimable summary statistics, e.g, the median, 6-month rate, 12-month rate, etc.

13.5 Analysis of Exploratory Endpoints

As per Section 1.3, the exploratory objectives are:

1) To determine the ORR in patients with locally advanced or metastatic TCC harboring a *TSC2* mutation.

2) To evaluate toxicity, PFS, and OS in *TSC2* mutation patients.

The analysis of the endpoints for Exploratory Objectives 1 and 2 will be similar to that proposed for Secondary Objectives 1 and 2, respectively.

13.6 Reporting and Exclusions

13.6.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with *MLN0128 (TAK-228)*

13.6.2 Evaluation of Response

All patients included in the study will be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data).

All eligible patients (with the possible exception of those who received no study medication) will be included in the main analysis of the response rate. Patients in response categories 4-9 will be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration will not result in exclusion from the analysis of the response rate.

All conclusions will be based on all eligible patients. Subanalyses may be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses will not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

REFERENCES

- Siegel, R., Naishadham, D. and Jemal, A. (2012), Cancer statistics, 2012. CA: A Cancer Journal for Clinicians, 62: 10–29. doi: 10.3322/caac.20138
- Howlader N, Noone AM, Krapcho M, Garshell J, Neyman N, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2010, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2010/, based on November 2012 SEER data submission, posted to the SEER web site, April 2013.
- National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology for Bladder Cancer; Version 1.2014;http://www.nccn.org/professionals/physician_gls/pdf/bladder.pdf. Accessed Feb 28 2014.
- Hans von der Maase, Lisa Sengelov, James T. Roberts, Sergio Ricci, Luigi Dogliotti, T. Oliver, Malcolm J. Moore, Annamaria Zimmermann and Michael Arning Long-Term Survival Results of a Randomized Trial Comparing Gemcitabine Plus Cisplatin, With Methotrexate, Vinblastine, Doxorubicin, Plus Cisplatin in Patients With Bladder Cancer;doi: 10.1200/JCO.2005.07.757 JCO July 20, 2005 vol. 23 no. 21 4602-4608
- Schenone, S., C. Brullo, F. Musumeci, et al. (2011). ATP-competitive inhibitors of mTOR: an update. Curr Med Chem. 18:2995-3014
- Seth A. Wander, Bryan T. Hennessy and Joyce M. Slingerland; Next-generation mTOR inhibitors in clinical oncology: how pathway complexity informs therapeutic strategy; J Clin Invest. 2011; 121(4): 1231–1241. doi:10.1172/JCI44145
- Feldman ME, Shokat KM. New inhibitors of the PI3K-Akt-mTOR pathway: insights into mTOR signaling from a new generation of Tor Kinase Domain Inhibitors (TORKinibs). Curr Top Microbiol Immunol. 2010; 347:241-62. doi: 10.1007/82_2010_64.
- Katayoun Jessen, Shunyou Wang, Linda Kessler, Xin Guo, Jeff Kucharski, Jocelyn Staunton, Lucy Lan, Marikka Elia, Josh Stewart, Jolene Brown, Liansheng Li, Katrina Chan, Michael Martin, Pingda Ren, Christian Rommel and Yi Liu; Molecular Cancer Therapeutics: December 2009; Volume 8, Issue 12, Supplement 1; INK128 is a potent and selective TORC1/2 inhibitor with broad oral antitumor activity.
- Linda Kessler, Shunyou Wang, Xin Guo, Jeff Kucharski, Jocelyn Staunton, Lucy Lan, Marikka Elia, Josh Stewart, Jolene Brown, Liansheng Li, Katrina Chan, Michael Martin, Pingda Ren, Katti Jessen, Christian Rommel, and Yi Liu; Cancer Research: April 15, 2010; Volume 70, Issue 8, Supplement 1; Abstract 4496: INK128, an orally active TORC1/2 kinase inhibitor, shows broad antitumor activity and enhances efficacy of cytotoxic as well as targeted agents
- B. Hassan; A. Akcakanat; S. Takafumi; K. Evans; F. Adkins; F. Meric-Bernstam; MTOR Inhibitor MLN0128 (TAK-228) Has Antitumor Efficacy In Cell Lines With Intrinsic And Acquired Rapamycin-Resistance; Academic Surgical Congress; Abstract ASC20130420; Feb 2013.

- Pradip KR. De, Yuliang Sun, Hui Wu, Christian Rommel, Liu Yi, Nandini Dey, and Brian Leyland-Jones (2012). Pre-clinical potency of INK128, a highly potent TORC1/2 kinase inhibitor in the HER2 amplified breast cancer model. Cancer Res. 72:2240 (Abstract 2240).
- Jeffrey R. Infante, Josep Tabernero, Howard A. Burris, Teresa Macarulla, Michael Martin, Yi Liu, Katti Jessen, Ethel Koczon, Christian Rommel, Pam M. Klein, Greg I. Berk, Lynne A. Bui, and Michael S. Gordon; Abstract 5588: A phase I, open label, dose escalation study of an oral mammalian target of rapamycin inhibitor INK128 administered once daily in patients with advanced malignancies; Cancer Research: April 15, 2012; Volume 72, Issue 8, Supplement 1. doi: 10.1158/1538-7445.AM2012-5588
- M. Ghobrial, MD, David Siegel, MD, PhD, Ravi Vij, MD, Jeffrey L Wolf, MD, Jesus G. Berdeja, MD, Jeffrey V. Matous, MD, Peter Lipman, PhD, Chirag Patel, MS, PhD, Trinh Le, Christian Rommel, PhD and Gregory Berk, MD; MLN0128 (TAK-228) (INK128), an Investigational Oral Dual TORC1/2 Inhibitor, in Patients (pts) with Relapsed or Refractory Multiple Myeloma (MM), Non-Hodgkin's Lymphoma (NHL), or Waldenstrom Macroglobulinemia (WM): Preliminary Results From a Phase 1 Dose-Escalation Study; Abstract 4038; ASH 2012; Abstract 4038 Session: 653)
- Josep Tabernero, Andres Cervantes, Michael S. Gordon, Elena G. Chiorean, Howard A. Burris, Teresa Macarulla, Alejandro Perez-Fidalgo, Michael Martin, Katti Jessen, Yi Liu, Trinh Le, Christian Rommel, Greg I. Berk, Lynne A. Bui, and Jeffrey R. Infante; Abstract CT-02: A phase I, open label, dose escalation study of oral mammalian target of rapamycin inhibitor INK128 administered by intermittent dosing regimens in patients with advanced malignancies; Cancer Research: April 15, 2012; Volume 72, Issue 8, Supplement 1 doi: 10.1158/1538-7445.AM2012-CT-02
- Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. Nat Rev Cancer 2004; **4**: 335–48.
- S Chan; targeting the mammalian target of rapamycin (mTOR): a new approach to treating cance; Br J Cancer. 2004 October 18; 91(8): 1420-1424
- Wen ZH, Su YC, Lai PL, Zhang Y, Xu YF, Zhao A, Yao GY, Jia CH, Lin J, Xu S, Wang L, Wang XK, Liu AL, Jiang Y, Dai YF, Bai XC. Critical role of arachidonic acid-activated mTOR signaling in breast carcinogenesis and angiogenesis. Oncogene. 2013 Jan 10; 32(2): 160-70. doi: 10.1038/onc.2012.47. Epub 2012 Feb 20.
- Chiang GG, Abraham RT. Targeting the mTOR signaling network in cancer. Trends Mol Med. 2007; 13(10): 433–442.
- Guertin, D.A. and Sabatini, D.M. (2007) Defining the Role of mTOR in Cancer. Cancer Cell 12, 9–22 4
- Sabatini, D.M. (2006) mTOR and cancer: insights into a complex relationship. Nat. Rev. Cancer 6, 729–734
- Weinberg, Robert A.; 2007; The biology of Cancer. . Garland Science: Taylor & Francis Group; New York City, NY; pgs 782-787

- Fingar DC, Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. Oncogene. 2004; 23:3151- 71. Review.
- Stokoe D, Stephens LR, Copeland T, et al. Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activa- tion of protein kinase B. Science 1997;277:567–70
- Stambolic V, Suzuki A, de la Pompa JL, et al. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell 1998; 95:29–39; Maehama T, Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3, 4, 5- trisphosphate. J Biol Chem. 1998;273:13375-8.).
- Bhaskar,P.T. and Hay,N.(2007)The two TORCs and Akt. Dev.Cell12, 487–502
- Sarbassov DD, Guertin DA, Ali SM, Sabatini DM; Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex; Science. 2005 Feb 18;307(5712):1098-101.
- Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J. Tuberous sclerosis complex gene products,Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. Curr Biol. 2003;13(15):1259–1268
- Long X, Lin Y, Ortiz-Vega S, Yonezawa K, Avruch J. Rheb binds and regulates the mTOR kinase; Curr Biol. 2005 Apr 26;15(8):702-13.
- Potter CJ, Pedraza LG, Xu T. Akt regulates growth by directly phosphorylating Tsc2. Nat Cell Biol. 2002;4(9):658–665;
- Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. Mol Cell. 2002;10(1):151–162;
- Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat Cell Biol. 2002; 4(9):648–657; Ken Inoki, Yong Li, Tianquan Zhu, Jun Wu and Kun-Liang Guan; TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signaling; Nat Cell Biol. 2002 Sep;4(9):648-57.
- Mehran S. Neshat, Ingo K. Mellinghoff, Chris Tran, Bangyan Stiles, George Thomas, Roseann Petersen, Philip Frost, James J. Gibbons, Hong Wu, and Charles L. Sawyers; Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRA/mTOR; 10314–10319; PNAS; August 28, 2001; vol. 98; no. 18
- Laura Magri, Marco Cambiaghi, Manuela Cominelli, Clara Alfaro-Cervello, Marco Cursi, Mauro Pala, Alessandro Bulfone, Jose Manuel Garcı`a-Verdugo, Letizia Leocani, Fabio Minicucci, Pietro Luigi Poliani, and Rossella Galli; Sustained Activation of mTOR Pathway in Embryonic Neural Stem Cells Leads to Development of Tuberous Sclerosis Complex-Associated Lesions Cell Stem Cell 9, 447–462, November 4, 2011
- Kwiatkowski DJ, Zhang H, Bandura JL, Heiberger KM, Glogauer M, el-Hashemite N, Onda H. Hum Mol Genet. A mouse model of TSC1 reveals sex-dependent lethality from liver hemangiomas, and up-regulation of p70S6 kinase activity in Tsc1 null cells. 2002 Mar 1;11(5):525-34;

- Ken Inoki and Kun-Liang Guan; Tuberous sclerosis complex, implication from a rare genetic disease to common cancer treatment; Human Molecular Genetics, 2009, Vol. 18;
- Zhang H, Cicchetti G, Onda H, Koon HB, Asrican K, Bajraszewski N, Vazquez F, Carpenter CL, Kwiatkowski DJ; Loss of Tsc1/Tsc2 activates mTOR and disrupts PI3K-Akt signaling through downregulation of PDGFR; J Clin Invest. 2003 Oct; 112(8): 1223-33.
- Jian-Hua Mao, Il-Jin Kim, Di Wu, Joan Climent,Hio Chung Kang, Reyno DelRosario and Allan Balmain; FBXW7 Targets mTOR for Degradation and Genetically Cooperates with PTEN in Tumor Suppression; Science. 2008 September 12; 321(5895): 1499–1502.
- Akhoondi S, Sun D, von der Lehr N, Apostolidou S, Klotz K, Maljukova A, Cepeda D, Fiegl H, Dafou D, Marth C, Mueller-Holzner E, Corcoran M, Dagnell M, Nejad SZ, Nayer BN, Zali MR, Hansson J, Egyhazi S, Petersson F, Sangfelt P, Nordgren H, Grander D, Reed SI, Widschwendter M, Sangfelt O, Spruck C. FBXW7/hCDC4 is a general tumor suppressor in human cancer Cancer Res. 2007 Oct 1; 67(19):9006-12.
- Markman B, Dienstmann R, Tabernero J. Targeting the PI3K/Akt/mTOR pathway--beyond rapalogs. Oncotarget. 2010 Nov;1(7):530-43.
- Carracedo, A., L. Ma, J. Teruya-Feldstein, et al. (2008). Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. J Clin Invest. 118:3065-3074
- Nardella C, Carracedo A, Alimonti A, Hobbs RM, Clohessy JG, Chen Z, Egia A, Fornari A, Fiorentino M, Loda M, Kozma SC, Thomas G, Cordon-Cardo C, Pandolfi PP. Differential requirement of mTOR in postmitotic tissues and tumorigenesis. Sci Signal. 2009 Jan 27; 2(55):ra2. doi: 10.1126/scisignal.2000189.
- Garcia JA, Danielpour D. Mammalian target of rapamycin inhibition as a therapeutic strategy in the management of urologic malignancies. Mol Cancer Ther 2008; 7: 1347–1354
- Chen M, Gu J, Delclos GL, Killary AM, Fan Z, Hildebrandt MA, Chamberlain RM, Grossman HB, Dinney CP, Wu X.; Genetic variations of the PI3K-AKT-mTOR pathway and clinical outcome in muscle invasive and metastatic bladder cancer patients. Carcinogenesis. 2010 Aug; 31(8): 1387-91. doi: 10.1093/carcin/bgq110. Epub 2010 Jun 7.
- Platt FM, Hurst CD, Taylor CF, Gregory WM, Harnden P, Knowles MA; Spectrum of phosphatidylinositol 3-kinase pathway gene alterations in bladder cancer. Clin Cancer Res. 2009 Oct 1; 15(19): 6008-17. doi: 10.1158/1078-0432.CCR-09-0898. Epub 2009 Sep 29
- Nick Hornigold, Jayne Devlin, Alison M Davies, Joanne S Aveyard, Tomonori Habuchi and Margaret A Knowles; Mutation of the 9q34 gene TSC1 in sporadic bladder cancer; Oncogene (1999) 18, 2657 ± 2661.
- Habuchi T, Devlin J, Elder PA, Knowles MA. Detailed deletion mapping of chromosome 9q in bladder cancer: evidence for two tumour suppressor loci. Oncogene 1995; 11:1671–4

- Margaret A. Knowles, Tomonori Habuchi, Wendy Kennedy, et al.; Mutation Spectrum of the 9q34 Tuberous Sclerosis Gene TSC1 in Transitional Cell Carcinoma of the Bladder Cancer Res 2003; 63:7652-7656. Published online November 21, 2003
- Louis S. Pymar, Fiona M. Platt, Jon M. Askham, Ewan E. Morrison and Margaret A. Knowles; Bladder tumour-derived somatic TSC1 missense mutations cause loss of function via distinct mechanisms; Human Molecular Genetics, 2008, Vol. 17, No. 13 2006–2017 doi: 10.1093/hmg/ddn098
- Sjödahl G, Lauss M, Gudjonsson S, Liedberg F, Halldén C, Chebil G, Månsson W, Höglund M, Lindgren D; A systematic study of gene mutations in urothelial carcinoma; inactivating mutations in TSC2 and PIK3R1; PLoS One. 2011 Apr 14; 6(4):e18583. doi: 10.1371/journal.pone.0018583.
- David Neal Franz, Jennifer Leonard, Cynthia Tudor, Gail Chuck, Marguerite Care, Gopalan Sethuraman, Argirios Dinopoulos, George Thomas, and Kerry R. Crone; Rapamycin Causes Regression of Astrocytomas in Tuberous Sclerosis Complex; Ann Neurol 2006; 59:490–498
- Darcy A. Krueger, Marguerite M. Care, Katherine Holland, Karen Agricola, Cynthia Tudor, Prajakta Mangeshkar, Kimberly A. Wilson, Anna Byars, Tarek Sahmoud, and David Neal Franz,; Everolimus for Subependymal Giant-Cell Astrocytomas in Tuberous Sclerosis N Engl J Med 2010; 363:1801-1811.
- E. Seront, S. Rottey, B. Sautois, J. Kerger, L. A. D'Hondt, V. Verschaeve, J.-L. Canon, C. Dopchie, J. M. Vandenbulcke, N. Whenham, J. C. Goeminne, M. Clausse, D. Verhoeven, P. Glorieux, S. Branders, P. Dupont, J. Schoonjans, O. Feron & J.-P. Machiels; Phase II study of everolimus in patients with locally advanced or metastatic transitional cell carcinoma of the urothelial tract: clinical activity, molecular response, and biomarkers; Ann Oncol. 2012 Oct; 23(10): 2663-70. Epub 2012 Apr 3
- The Cancer Genome Atlas Research N: Comprehensive molecular characterization of urothelial bladder carcinoma. Nature advance online publication, 2014
- Gopa Iyer, Aphrothiti J. Hanrahan, Matthew I. Milowsky, Hikmat Al-Ahmadie, Sasinya N. Scott, Manickam Janakiraman, Mono Pirun, Chris Sander, Nicholas D. Socci, Irina Ostrovnaya, Agnes Viale, Adriana Heguy, Luke Peng, Timothy A. Chan, Bernard Bochner, Dean F. Bajorin, Michael F. Berger, Barry S. Taylor, David B Solit. Genome Sequencing Identifies a Basis for Everolimus Sensitivity; Science. 2012 Oct 12; 338(6104):221. doi: 10.1126/science.1226344. Epub 2012 Aug 23
- Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J; New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1); Eur J Cancer. 2009 Jan;45(2):228-47. doi: 10.1016/j.ejca.2008.10.026.
- Guo Y, Chekaluk Y, Zhang J, Du J, Gray NS, Wu CL, Kwiatkowski DJ; TSC1 involvement in bladder cancer: diverse effects and therapeutic implications; J Pathol. 2013 May;230(1):17-27. doi: 10.1002/path.4176. Epub 2013 Mar 21.

- Hintze, J. (2011). PASS 11. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com

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

Not applicable.

APPENDIX C INFORMATION ON POSSIBLE DRUG INTERACTIONS

Information on Possible Interactions with Other Agents for Patients and Their Caregivers and Non-Study Healthcare Team

The patient _____ is enrolled on a clinical trial using the experimental agent **MLN0128 (TAK-228).** *This clinical trial is sponsored by the National Cancer Institute. This form is addressed to the patient, but includes important information for others who care for this patient.*

MLN0128 (TAK-228) may interact with many drugs that are processed by your liver. Because of this, it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the-counter remedy), or herbal supplements such as St. John's wort.

Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you and keep the attached information card in your wallet**. These are the things that you and they need to know:

MLN0128 (TAK-228) may interact with (a) certain specific enzyme(s) in your liver.

- The enzyme(s) in question are CYP3A4, CYP2C9, and CYP2C19. MLN0128 (TAK-228) is broken down by this enzyme in order to be cleared from your system.
- MLN0128 (TAK-228) must be used very carefully with other medicines that need these liver enzymes to be effective or to be cleared from your system.
- Other medicines may also affect the activity of the enzyme.
    - Substances that increase the enzyme's activity ("inducers") could reduce the effectiveness of the drug, while substances that decrease the enzyme's activity ("inhibitors") could result in high levels of the active drug, increasing the chance of harmful side effects.

- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drug in this category.
- Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors or substrates of CYP3A4, CYP2C9, and CYP2C19
- Your prescribers should look at this web site http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#cypEnzymes) or consult a medical reference to see if any medicine they want to prescribe is on a list of drugs to avoid.
- Please be very careful! Over-the-counter drugs have a brand name on the label—it's usually big and catches your eye. They also have a generic name—it's usually small and located above or below the brand name, and printed in the ingredient list. Find the generic name and determine, with the pharmacist's help, whether there could be an adverse interaction.

- Be careful:
    - If you take acetaminophen regularly: You should not take more than 3 grams a day if you are an adult or 2.4 grams a day if you are older than 65 years of age. Read labels carefully! Acetaminophen is an ingredient in many medicines for pain, flu, and cold.
    - If you drink grapefruit juice or eat grapefruit: Avoid these until the study is over.
    - If you take herbal medicine regularly: You should not take St. John's wort while you are taking MLN0128 (TAK-228)
- You should **not take proton pump inhibitors,** a class of medicines to reduce stomach acid, while taking MLN0128 (TAK-228). Examples include omeprazole, esomeprazole, or pantoprazole.
- Unless started more than 30 days ago, you should not start taking any bisphosphonate therapy, a class of medications to prevent of bone fractures, while taking MLN0128 (TAK-228).

Other medicines can be a problem with your study drugs.

- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

- Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you. Your study doctor's name is

_____

and he or she can be contacted at

_____

INFORMATION ON POSSIBLE DRUG INTERACTIONS

You are enrolled on a clinical trial using the experimental agent MLN0128 (**TAK-228)**. This clinical trial is sponsored by the NCI. **MLN0128 (TAK-228)** may interact with drugs that are processed by your liver. Because of this, it is very important to:

- Tell your doctors if you stop taking regular medicine or if you start taking a new medicine.
- Tell all of your prescribers (doctor, physicians' assistant, nurse practitioner, pharmacist) that you are taking part in a clinical trial.
- Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

MLN0128(TAK-228) is metabolized by specific liver enzymes called CYP3A4, CYP2C9, and CYP2C19, and must be used very carefully with other medicines that interact with these enzymes.

- Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors or substrates of called CYP3A4, CYP2C9, and CYP2C19.
- Before prescribing new medicines, your regular prescribers should go to http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#cypEnzymes) for a list of drugs to avoid, or contact your study doctor.

APPENDIX D: DIARY FOR MLN0128 (TAK-228)

Date: _____Cycle: _____ Subject ID: _____Subject Initials: ____ ____

* Please take capsule(s) approximately the same time on each scheduled day. You will take _____ capsule(s) each day. Swallow capsules whole with water. Refrain from eating and drinking (except water and prescribed medications) for 2 hours before and 1 hour after each dose. Stay hydrated by drinking at least 2-3 cups of water each day.

*** Please bring this diary and capsule bottle with you to each appointment**.

* If you miss a dose and realize it within 12 hours, please take the dose on an empty stomach. Do not take 2 doses 12 hours or less apart. If you vomit after dosing, please note in the Comment column below, do not take another dose.

*** Always** contact your study doctor **before** taking any new medications.

* Do **not** eat grapefruit or drink grapefruit juice while you are on study. Day in Cycle	Date of Dosing	Time of Dosing	Number of capsules taken	Did you vomit after dosing? If so, please note the time. Other comments:	Fasting Blood Glucose
D1					
D2					
D3					
D4					
D5					
D6					
D7					
D8					
D9					
D10					
D11					
D12					
D13					
D14					
D15					
D16					
D17					
D18					
D19					
D20					
D21					
D22					
D23					
D24					
D25					
D26					
D27					
D28					

Patient's Signature or Initials: _____ Date: _____

This diary has been reviewed by:

Signature: _____Date _____

Printed Name: _____

APPENDIX E: NEW YORK HEART ASSOCIATION (NYHA) CLASSIFICATION OF CARDIAC DISEASE

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Source: The Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th Ed. Boston, MA: Little, Brown & Co; 1994:253-256.

APPENDIX F: LIST OF RELEVANT CYTOCHROME P450 INHIBITORS AND INDUCERS

MLN0128 (TAK-228) is metabolized by CYP2C19, CYP3A4, and CYP2C9. Thus, **strong** inhibitors and/or inducers of CYP2C19, CYP3A4 or CYP2CP are **prohibited** on study. Strong inhibitors and inducers of CYP2C19 and CYP3A4 are listed in the table below. There are no known strong specific inhibitors or inducers of CYP2C9. Investigators must check the link to the FDA website below for any updates. Note the list of these medications is from the Tables 3-2 and 3-3 of the FDA website. They were updated on 9/26/2016.

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#cypEnzymes

Moderate inhibitors and/or inducers of CYP2C19, CYP3A4 and CYP2C9 may be used on study with caution unless they are strong inhibitors and/or inhibitors of these isozymes. Check the FDA website link above for the information.

Strong Inhibitors and Strong Inducers of CYP2C9, CYP2C19, and CYP3A4

Strong Inhibitors	Strong Inducers
boceprevir	carbamazepine
cobicistat	enzalutamide
conivaptan	mitotane
clarithromycin	phenytoin
danoprevir and ritonavir	Rifampin
diltiazem	Ritonavir
elvitegravir and ritonavir	St. John's wort
fluconazole	
fluoxetine	
fluvoxamine	
idelalisib	
indinavir and ritonavir	
itraconazole	
ketoconazole	
lopinavir and ritonavir	
nefazodone	
nelfinavir	
paritaprevir and ritonavir and (ombitasvir and/or dasabuvir)	
posaconazole	
ritonavir	
saquinavir and ritonavir	
telaprevir	
tipranavir and ritonavir	
troleandomycin	

voriconazole
ticlopidine
Fruits and juice
grapefruit and juice

Sources:
http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#cypEnzymes