IST-SAR-081 Phase II study of TAK-228 (MLN0128) in soft tissue sarcomas with dysregulation of the mTOR pathway

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STUDY OVERVIEW DIAGRAM

Schema

Patients with complex genomic sarcomas

- LMS, myxofibrosarcoma, pleomorphic liposarcoma, pleomorphic rhabdomyosarcoma, MPNST, angiosarcoma, HGUPS, extraskeletal osteosarcoma
- Dysregulation of the PI3K/AKT/mTOR pathway (protein or genomic)
- Unresectable or metastatic
- Failed at least 1 prior therapy

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Treatment

- TAK-228 3 mg daily
- 21 day cycle (± 3 days)

↓

Disease Assesment

- 6, 12, 18 weeks and then every 12 weeks (± 3 days)

↓

Continue treatment until

- Disease progression
- Unacceptable Toxiciy
- Patient consent withdrawal

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Follow-up

- Follow-up every 3 months for the first 2 years, then every 6 months for 2 years, then annually for study related survival

STUDY CALENDAR

	Step 1 Screening[b]	Step 2 Screening	Every Cycle (- 3 days)	End of Therapy[m]	Follow-up
Informed consent & HIPAA[a]	X				
Medical history	X				
Physical exam	X		X	X	
Concurrent Meds	X		X	X	
Inclusion/Exclusion	X				
Biopsy/Archival tissue and tumor analysis		X[c]			
Vital signs[d]	X		X	X	
Height	X				
Weight	X		X	X	
Performance status	X		X	X	
PT/INR, APTT	X		X	X	
CBC w/diff	X		X	X	
Complete metabolic profile[e]	X		X	X	
Fasting blood glucose[f]	X		X	X	
Fasting lipid profile[g]	X		X	X	
HbA1C	X		C1D1 and then every 3 months while on therapy (± 7 days)		
β-HCG[h]	X		X		
TSH, free T4	X				
Urinalysis	X		X	X	
EKG[i]	X		X	X	
Adverse Events	X		X	X	
Radiologic Evaluation: CT or MRI[j]	X		At week 6, 12, 18 and then every 12 weeks (± 3 days)	X[n]	X[o]
Tumor Measurements[k]	X				X[o]
Survival Status					X[p]
TAK-228[l]			X		

a)Informed consent must be signed prior to screening procedures.
b) Pre-study H&P, labs, EKG, Tumor measurements and radiologic evaluations must be completed ≤ 28 days before study drug initiation.
c)Archival tissue - Tumor must have PTEN loss either by IHC or CMA or PIK3CA/PTEN mutation. See section 9.2.2.6. Patients must be willing and able to provide fresh tumor for biopsy and biomarker analysis if archival tissue is not sufficient. In the event that a patient has had tumor analyzed for PTEN status through commercial means, their eligibility and need for additional tissue will be determined on a case by case basis.
d) Vital signs include temperature, respiratory rate, heart rate and blood pressure. In addition, O2 saturation must be measured by office pulse oximetry during screening and at the beginning of every cycle..
e) Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, sodium, total protein, SGOT [AST], SGPT [ALT].
f) Patients will also monitor pre-dose fasting blood sugar daily (monitor provided). 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 glucose testing may be reduced to once weekly if the investigator approves.
g) Includes fasting triglycerides and total cholesterol.
h) Serum pregnancy test (women of childbearing potential) must be completed ≤ 72 hours before beginning treatment.
i) EKG should be completed at screening, end of treatment visit and as needed during treatment.
j) Anatomical imaging should include CT of the chest, abdomen and pelvis. Patients who cannot have CT may have an MRI. The same imaging modality should be used at baseline and follow-up.
k) Response to treatment will be measured using RECIST 1.1 criteria.
l) TAK-228 3 mg daily every 21 days. Patients should refrain from eating for 2 hours before and 1 hour after each dose. Subjects should be encouraged to drink at least 20 ounces of fluid per day.
m) End of treatment visit will occur when all study related toxicities are Grade 1 or lower (or baseline if present at study entry) or deemed to be irreversible.
n) Disease assessment will be performed at the end of therapy if clinically feasible.
o) Subjects who discontinue treatment for reasons other than disease progression should continue to have radiographic assessments of disease at least every 3 months until documented disease progression.
p) Follow-up every 3 months for the first 2 years, then every 6 months for 2 years, then annually for study survival.

CONTENTS

1.0 Background and Study Rationale

1.1. Scientific Background

1.1.1. Disease Under Treatment

Sarcomas are a rare, heterogeneous group of mesenchymal tumors. In 2015, there were an estimated 14,909 cases of newly diagnosed bone and soft tissue cancers associated with 6,360 deaths in the United States. Soft tissue sarcomas (STS) can be classified according to the genetic alterations involved in their development: those with oncogenic somatic mutations [e.g. gastrointestinal stromal tumors (GIST)], those with DNA copy number alterations (e.g. dedifferentiated liposarcomas), and those with recurrent chromosomal translocations resulting in abnormal fusion proteins (e.g. synovial sarcomas). More commonly, sarcomagenesis is a result of complex chromosomal abnormalities, as in the case of leiomyosarcomas (LMS), myxofibrosarcomas, pleomorphic liposarcomas, pleomorphic rhabdomyosarcomas, malignant peripheral nerve sheath tumors (MPNST), angiosarcomas, high grade undifferentiated pleomorphic sarcomas and extraskeletal osteosarcomas.[1] These tumors account for 50% of STS and display gains and losses of numerous chromosomes or chromosome regions. Many share recurrent alterations that play a role in progression of disease and/or metastases.

With over 100 sarcoma subtypes, a single therapeutic strategy for this group of cancers is unfeasible. For patients with advanced STS, the selection of therapy is based on the specific sarcoma subtype as well as the patient's fitness to receive aggressive chemotherapy. With this approach, median overall survival for patients with STS remains under two years.[2] Broadly, the most effective and utilized agents in advanced sarcoma include the anthracyclines, with or without the addition of alkylating agents and the combination of gemcitabine and docetaxel. Though targeted therapy has significantly improved survival in patients with GIST, the broad use of targeted agents in sarcoma has been a challenge. Pazopanib a multikinase angiogenesis inhibitor is the only targeted agent approved for general use in STS after a phase III clinical trial demonstrated a progression free survival benefit when compared with placebo (4.6 versus 1.5 months, $P < 0.0001$).[3]

The PI3K/AKT/mTOR pathway is of great interest in sarcoma and cancer in general. This pathway is involved in cell proliferation, tumor angiogenesis, and abnormal cellular metabolism and its inhibition is an attractive therapeutic target. PTEN is a tumor suppressor gene which regulates PI3K activation. PI3K activates AKT which in turn activates mTOR, leading to increased cell proliferation and reduction of apoptotic mechanisms. PTEN loss has been been linked with the development of some complex genomic sarcomas.[4-7] Decreased protein expression of PTEN has been noted in 29%-68% of STS with complex genomics.[6,8,9]

First generation mTOR inhibitors such as rapamycin, temsirolimus and everolimus are specific partial inhibitors of mTORC complex 1 signaling, and therefore do not inhibit mTOR complex 2 (mTORC2). Preclinically, rapamycin and its analogues have inhibited growth of a variety of sarcoma cell lines including: rhabdomyosarcoma, osteosarcoma and Ewing's sarcoma. Disappointingly, we and others have failed to show benefit in the clinic using first generation mTOR inhibitors patients with sarcoma.[10-12] A phase II study of ridaforlimus (AP23573; Ariad Pharmaceuticals) in advanced refractory bone and STS demonstrated a clinical benefit rate at 16

weeks of 29%, with 4 patients achieving a partial response (two with osteosarcoma, one with spindle cell sarcoma, and one with malignant fibrous histiocytoma).[13] The most significant side effects were mucositis, rash, hyperlipidemia, fatigue and thrombocytopenia. In the subsequent maintenance study of the oral formulation of this agent, (Sarcoma mUlti-Center Clinical Evaluation of the Efficacy of riDaforolimus, SUCCEED), patients who achieved stable disease or better after chemotherapy were randomized to ridaforlimus or placebo. There was a statistically significant PFS improvement of 3.1 weeks (17.7 versus 14.6) favoring the ridaforlimus arm.[14] The lack of significant clinical benefit for these patients resulted in the FDA's denial of regulatory approval for this agent. A phase II study of another agent, temsirolimus, given weekly to patients with advanced chemotherapy naïve STS also failed to meet its primary endpoint. Two patients (leiomyosarcoma, fibrosarcoma) had a partial response and median time to progression was 2.0 months.[10] In refractory bone and STS, the response rate to everolimus was also dissapointingly low, although toxicity was managable.[15] Of importance, none of these studies required patients to have tumors with documented dysregulation of the mTOR pathway for eligibility.

PTEN loss, though a relatively frequent event in complex genomic sarcomas, appears to occur in approximately only 46% of these tumors. Therefore, preselection for patients whose tumors specifically have aberrant signaling of this pathway may lead to better success in clinical trials. In addition, RICTOR a major component on mTORC2 has been found to be overexpressed in some sarcomas, thus also likely playing an important role in sarcomagenesis.[9] Therefore, it is our hypothesis that targeting both mTORC1 and mTORC2 with TAK-228, in sarcomas specifically with dysregulation of the PI3-kinase pathway, will translate into clinical benefit for patients.

1.1.2. TAK-228

Millennium has developed TAK-228, which is a novel, highly selective, orally bioavailable adenosine 5' triphosphate (ATP)-competitive inhibitor of the serine/threonine kinase referred to as the mechanistic target of rapamycin (mTOR). TAK-228 (formerly INK128) targets 2 distinct mTOR complexes, mTORC1 and mTORC2.

TAK-228 selectively and potently inhibits mTOR kinase (IC_{50} = 1.1 nM), inhibits mTORC1/2 signaling, and prevents cellular proliferation. The mTOR is a kinase that regulates cell growth, translational control, angiogenesis, and cell survival by integrating nutrient and hormonal signals. mTOR kinase plays a key role in several pathways that are frequently dysregulated in human cancer.[16] mTORC1 is best known as a key regulator of protein translation through phosphorylation of 4EBP1 (the eukaryotic translation Initiation Factor 4E-binding Protein 1) and ribosomal protein S6 (known as S6) kinase. mTORC2 is best known for its ability to fully activate protein kinase B (AKT) by phosphorylation on the S473 site, which regulates proliferation and survival pathways.[17]

The mTORC is an important therapeutic target that is a key intracellular point of convergence for a number of cellular signaling pathways. Inhibiting mTOR may inhibit abnormal cell proliferation,tumor angiogenesis, and abnormal cellular metabolism, thus providing the rationale for mTOR inhibitors as potential agents in the treatment of a number of indications including solid tumor and hematological malignancies, as either monotherapy or in combination with other chemotherapeutic agents. Like rapamycin, several newly approved rapalogs (temsirolimus and

everolimus) are specific and allosteric inhibitors of mTORC1, and only partially inhibit mTORC1 signaling pathways. They do not directly inhibit mTORC2, which has shown to be an emerging target in cancer research. TAK-228 was developed to address the incomplete inhibition of the mTOR pathway by rapalogs by targeting both mTORC1 and mTORC2.

TAK-228 is being developed for both oncology and non-oncology indications. In oncology, TAK-228 is being investigated as a treatment for advanced solid tumors and hematologic malignancies, either as monotherapy or in combination with chemotherapy, other molecularly targeted therapies, or antihormonal agents. Non-oncology indications being investigated include fibrotic and inflammatory diseases.

2.0 NonClinical Summary

2.1. Pharmacology

TAK-228 selectively and potently inhibits mTOR kinase (the concentration inhibiting 50% of enzyme activity [IC_{50}] is 1.1 nM), inhibits mTORC1/2 signaling, and prevents cellular proliferation.

TAK-228 inhibited phosphorylation of downstream modulators of mTORC1and mTORC2 in human U87 glioblastoma tumor xenograft models in mice and showed strong tumor growth inhibition (TGI) at tolerable oral (PO) doses in all 8 xenograft models tested (see IB Ed8 for details).

2.2. Safety Pharmacology

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

2.3. Drug Metabolism and Pharmacokinetics

TAK-228 was rapidly absorbed after PO administration to mice, rats, dogs, and monkeys, with high oral bioavailability. [^{14}C]TAK-228 was rapidly and widely distributed throughout the body in Long-Evans rats; radioactivity was eliminated from most tissues at 48 hours postdose. 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. TAK-228 distributed mainly to the plasma of human blood. There was no obvious concentration-dependent red blood cell (RBC) distribution of TAK-228 in human blood.

TAK-228 did not inhibit P-glycoprotein, but did inhibit breast cancer-resistance protein (BCRP), organic cation transporter (OCT)1 and OCT2.

M1, the single metabolite (monohydroxylation product) observed in human microsomal incubations, was also observed in rats and monkeys. Recently completed in vitro metabolism experiments in human hepatocytes using ^{14}C-labeled TAK-228 suggest that TAK-228 is

metabolized primarily via CYP1A2 (approximately 31%-40%), with a minor contribution from CYP3A4 (approximately 11%-22%). These data suggest that TAK-228 is also metabolized by direct glucoronidation (approximately 22%) and an unidentified non-uridine diphosphate glucoronysyl transferase pathway (approximately 18%). The new data differ from the previous in vitro CYP phenotyping data obtained using recombinant CYP enzymes, which suggested the involvement of CYP2C9 (approximately 19%), CYP2C19 (approximately 28%), and CYP3A4 (approximately 28%) in TAK-228 metabolism. In addition, physiologically based PK modeling and simulation using the new metabolism data for TAK-228 suggest that the risk for a metabolism-based drug-drug interaction with TAK-228 appears to be low. Therefore, strong CYP1A2 inhibitors and CYP inducers should be administered with caution and at the discretion of the investigator during the study.

Oral administration of 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]).

2.4. Toxicology

The toxicologic profiles obtained in the Good Laboratory Practice (GLP)-compliant and non-GLP-compliant studies in rats and monkeys were generally consistent with pharmacologic inhibition of mTORC1/2 activity. Observed toxicities were mostly consistent between sexes. TAK-228 repeat-dose GLP studies include completed 28-day and preliminary 3-month toxicology studies in rat and monkeys, and embryo-fetal studies in rats and rabbits.

The primary dose-limiting toxicities (DLTs) of TAK-228 in rats and monkeys were secondary to an exaggerated pharmacologic response and consisted of body weight loss and associated clinical observations that included hunched posture, dehydration, gastrointestinal (GI) distress and decreased activity, appetite, and body temperature. In addition to the previously mentioned effects, a single monkey in the 3-month toxicology study demonstrated a DLT of skin toxicity characterized as progressive acanthosis. The highest exposures tolerated in the preliminary 3-month rat and monkey toxicology studies were 1233 and 194 ng·hr/mL, respectively.

Adverse effects in rats 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. The alveolar histiocytosis was only present in the 28-day rat study and was absent in the 3-month rat study. Both retinal dysplasia and alveolar histiocytosis are thought to be potential background findings. The pancreatic islet degeneration and fibrosis, as well as the other findings of lens fiber degeneration, pituitary atrophy, and liver vacuolation, were consistent with the mechanism of action (MOA) and effects observed with other rapalogs. The microscopic findings observed in the testes, epididymides, and eyes in the 28-day and/or 3-month rat studies were not resolved after a 14-day recovery period, while partial to complete

resolution was seen in the pancreas, adrenal gland, pituitary, liver, lungs, thymus, nonglandular stomach, and bone marrow.

The adverse effects in monkeys included hunched posture, dehydration, GI distress, and decreased activity, appetite, and body weight; increased glucose and insulin; lymphoid and bone marrow depletion; adrenal hypertrophy/hyperplasia; pancreatic and salivary gland acinar cell secretory depletion; neutrophilic inflammation of GI tract with occasional erosion and ulceration, skin effects including ulceration, epidermal hyperplasia, acanthosis and hyperkeratosis; and adipose tissue depletion. Additionally, there were sporadic inflammatory findings among some animals that were of uncertain association to the test article. The findings in the pancreas, adrenal glands, and salivary glands may have been related to a stress response or reduced food consumption. The findings observed in repeat-dose monkey studies were generally reversible after a 14-day recovery period.

The findings in rat and monkey repeat-dose toxicology studies with TAK-228, including bone marrow and lymphoid depletion; GI, liver, pancreas, and skin effects; and effects on glucose and insulin levels, can be monitored in clinical trials. The toxicities seen in the repeat-dose toxicology studies, such as GI effects and glucose and insulin increases, are consistent with the treatment-emergent adverse events (TEAEs), including mucositis and hyperglycemia, observed to date in patients receiving TAK-228.

Rat and rabbit range-finding embryo-fetal studies demonstrated teratogenic, fetotoxic, and abortive effects with TAK-228. Embryo-fetal lethality and/or teratogenic effects have been reported with the mTORC1 inhibitors rapamycin and the rapalogs.

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. An in vitro chromosomal aberration assay with TAK-228 in human peripheral blood lymphocytes (PBLs) was positive for chromosomal aberrations in the presence and absence of metabolic activation. However, the weight of evidence from the combined results of a negative mutagenicity assay and negative genotoxicity assessments in 2 in vivo assays in 3 tissues (bone marrow, liver, and duodenum) demonstrate that TAK-228 does not present a genotoxic risk.
TAK-228 was negative for phototoxicity in the 3T3 fibroblast assay.

3.0 Summary of Effects in Humans

TAK-228 is in clinical development as a single agent in 3 phase 1 studies including study INK128-01 in patients with advanced solid malignancies, study INK128-002 in patients with multiple myeloma [MM], non-Hodgkin lymphoma [NHL] and Waldenström macroglobulinemia [WM]) and study C31002 to measure the effect of TAK-228 on QTc interval in patients with advanced solid malignancies. It is also being investigated in combination with paclitaxel (with or without trastuzumab) in patients with advanced solid tumors (Ph1 study INK128-003), and in combination with exemestane or fulvestrant in women with ER+/HER2– (estrogen receptor-positive /human epidermal growth factor receptor 2 protein-negative) advanced or metastatic breast cancer (Ph1b/2 study C31001)

TAK-228 dosing regimens tested thus far include QD, QW, QD×3days per week (once daily for 3 consecutive days followed by a 4-day dosing holiday every week), and QD×5days per week (once daily for 5 consecutive days followed by a 2-day dosing holiday every week).

Please note that the data described in this section (sections 3.1 and 3.2) was obtained with the original unmilled TAK-228 active pharmaceutical ingredient (API); current manufacturing process produces milled TAK-228 API (see section 4).

3.1. Pharmacokinetics

Overall, pharmacokinetic (PK) data from Studies INK128-001, INK128-002, and INK128-003 indicate that TAK-228 exhibits fast oral absorption (time to reach C_{max} [t_{max}], 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) and under any of 4 tested dosing regimens. The PK of TAK-228 was generally consistent, with no appreciable differences across the clinical studies that measured PK. Neither paclitaxel nor TAK-228 appeared to alter the PK of the other agent when co-administered.

3.2. 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 TAK-228 (see section 5.3.1.1 of the IB Ed 8)

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.

3.2.1. Study INK128-001

Study INK128-001 is a phase 1, first-in-human, dose-escalation study of single-agent TAK-228 administered to patients diagnosed with advanced solid malignancies, including, but not limited to, colorectal, renal, endometrial, and urothelial tumors. Four dosing schedules are being evaluated (QD, QW, QD×3days per week, and QD×5days per week). Each schedule is administered in 28-day cycles.

As of 09 December 2014, a total of 194 patients had been enrolled. Median age at baseline was 60 years (range, 24-89 years), most (95%) patients are white, and 54% are women. As of data cutoff, 42% had received ≥ 1 dose of TAK-228 in 2 treatment cycles, while 8% had entered 3 cycles, and 10% had entered 4 cycles. The highest number of cycles that had been initiated as of data cutoff was 46.

The maximum tolerated doses (MTDs) for the 4 schedules investigated in INK128-001 were determined to be 6 mg for QD dosing, 16 mg for QD×3days per week dosing, 10 mg for

QD×5days per week dosing, and 40 mg for QW dosing.

Deaths
As of 09 December 2014, a total of 7 patients in this study had died within 30 days of their last dose of study drug as reported to the clinical database. One death was due to ventricular fibrillation and cardiac arrest, 1 was due to pleural effusion, 1 was due to sepsis, 1 was due to respiratory failure, and the remainder was due to disease progression. The event of ventricular fibrillation and cardiac arrest was the only case considered study drug-related; details are provided in section 5.3.1.1. of the IB Ed8.

Serious Adverse Events
As of the clinical database cutoff date, treatment-emergent SAEs had been reported for 82 patients (42%) in this study. The most commonly reported (≥ 4 patients, overall) preferred terms were stomatitis in 7 patients (4%), pneumonia in 6 patients (3%), abdominal pain or anemia in 5, each (3%), and vomiting, asthenia, or renal failure acute in 4, each (2%).

Treatment-Emergent Adverse Events
Overall, ≥ 1 TEAE was reported for 194 (100%) of the patients. Across the dosing groups, the most commonly reported TEAEs were nausea or hyperglycemia, which were each reported in 125 patients (64%). The second most common TEAE was vomiting (54% of patients), followed by fatigue (51%).

Across all dosing groups, ≥ 1 TEAE of severity ≥ Grade 3 had been reported for 68% of treated patients as of the clinical data cutoff date. Severity ≥ Grade 3 TEAEs, regardless of causality, that were reported in ≥ 5% of patients as of the data cutoff were hyperglycemia (14% of patients), fatigue or hypophosphatemia (8% each), asthenia (7%), anemia or stomatitis (6% each), and lymphopenia or nausea (5% of patients each).

Events leading to study discontinuation
Of the 194 patients treated in Study INK128-001 as of the clinical data cutoff, 110 (57%) discontinued because of disease progression, 20 (10%) withdrew consent, and 15 (8%) were lost to follow-up or discontinued for other reasons.

A total of 68 AEs led to study discontinuation among 35 patients (18%). Of these events, 32 (47%), including 16 nonserious AEs, were reported as severity Grade 3, and 6 SAEs were Grade 5. No Grade 4 events were reported as resulting in study discontinuation. Most (71%) events were considered study drug-related and had resolved as of the data cutoff date.

A total of 12 preferred terms were reported as leading to discontinuation for >1 patient, including rash (9 patients, including the terms maculopapular [5 patients], rash [2], and rash erythematous or rash pruritic [1 each]), nausea or stomatitis (7 patients each), pruritus or pruritus generalized (4 patients total), and asthenia, fatigue, renal failure/renal failure acute (3 patients, each). Events reported in 2 patients included hyperglycemia, pain or pain in extremity, performance status decreased, and vomiting.

3.2.2. Study INK128-002

Study INK-002 is a completed phase 1, open-label, dose-escalation study of oral TAK-228 administered as a single agent in patients with relapsed or refractory hematologic malignancies (MM or non-Hodgkin lymphoma, including WM). A total of 39 patients received TAK-228 in 1 of 2 regimens: 26 patients received QD doses (range, 2-7 mg) and 13 patients were dosed on a QD×3days per week schedule (range, 9-12 mg). The MTD for the QD schedule was 4 mg. The MTD for the QD×3days per week schedule was 9 mg.

A total of 21 of the patients (54%) in this study were male and 87% were white. The median age at baseline was 61 years (range, 46-85 years).

Deaths
Two patients died during Study INK128-002. One death was due to a subdural hemorrhage, and the other was due to disease progression. Both events were considered by the investigator to be unrelated to TAK-228.

Serious Adverse Events
Treatment-emergent SAEs were reported in Study INK128-002 for 11 patients (28%). No SAE occurred in more than 1 patient. Overall, most SAEs were considered severity Grade 2 or 3. Grade 4 SAEs were reported in 2 patients: hyperviscosity syndrome and hyponatremia were reported in 1 patient in the 2-mg QD dose group (both events resolved); and acute renal failure was reported in 1 patient in the 12-mg QD×3days per week dose group (resolved with sequelae).

No SAEs were considered to be related to TAK-228 treatment, with the exception of 3 events that were reported in 1 patient. This patient experienced Grade 2 pneumonia on Day 58 that resolved without sequelae on Day 60. On Day 121, the same patient experienced SAEs of pneumonia (Grade 2) and hypoxia (Grade 3). The 3 events improved by Day 125 and were resolved as of Day 142. All 3 events were considered by the investigator to be related to TAK-228.

Treatment-Emergent Adverse Events
All patients in Study INK128-002 experienced at least 1 TEAE. Overall, nausea was the most frequently reported preferred term (in 56% of patients), followed by fatigue (49%), hyperglycemia (38%), thrombocytopenia (36%), and diarrhea (28%).

TEAEs of severity ≥ Grade 3 were reported in 24 patients (62%); of these, 18 patients (46%) experienced ≥ Grade 3 events that were considered related to study drug. The most common study drug-related ≥ Grade 3 TEAEs were thrombocytopenia (in 15% of patients) and fatigue (10%).

Events Leading to Study Discontinuation
Overall, a total of 20 patients (51%) in Study INK128-002 discontinued due to progressive disease, 11 patients (28%) withdrew consent, and 6 (15%) discontinued due to investigator decision or other reasons.

Most events leading to study discontinuation were considered nonserious. Fatigue was reported

as resulting in study discontinuation in 2 patients; all other events were reported as leading to study discontinuation in 1 patient only.

3.2.3. Study INK128-003

Study INK128-003 is a phase 1, open-label, dose-escalation study of oral TAK-228 administered in 4-week cycles in combination with paclitaxel in patients with advanced solid malignancies (lung, ovarian, endometrial, breast, pancreatic, prostate, etc).As of the clinical data cutoff date, the treatment period for the primary endpoint had completed and long-term treatment for 1 patient remained ongoing.

In this study, 67 patients received ≥ 1 study drug dose under 1 of 3 dosing schedules: QW; QD×3days per week; and QD×5days per week. With each regimen, paclitaxel 80 mg/m^2 was dosed on Days 1, 8, and 15 of each cycle. Patients who tested positive for HER2+ received the combination and also received trastuzumab QW.

At total of 57% of the patients are women and 93% are white. At baseline, the median age was 60 years (range, 21-81 years).

On the basis of dose escalation data, 8 mg of TAK-228 on the QD×3days per week schedule was selected for the dose expansion phase in patients with breast cancer. The QD×5days per week and QW schedules were abandoned before MTDs were declared, as they were viewed as being less convenient relative to the QD×3days per week schedule from the perspective of administering the paclitaxel and trastuzumab combination.

Overall in the dose expansion phase, patients entered a median of 3.0 treatment cycles (range, 1-19 cycles) and a mean (SD) of 5.6 (6.07) cycles. The overall median duration of exposure was 7.5 weeks, with a duration over 2-fold greater (11.1 weeks) in the TAK-228 8 mg QD×3days per week HER2- treatment group relative to the TAK-228 8 mg QD×3days per week HER2+ plus trastuzumab group (5.2 weeks). The median cumulative dose was 189.0 mg. Across treatment groups, patients received approximately 75% of their planned dose of TAK-228.

Deaths

As of the clinical data cutoff date, 9 patients in this study had died within 30 days of administration of their last dose of study drug. Of these patients, 6 died due to disease progression, 1 died due to failure to thrive, 1 died due to enlarging tumor mass causing tracheal compression, and 1 died due to pneumonia. None of the events were considered related to TAK-228.

Serious Adverse Events

As of the clinical data cutoff date, 55 SAEs had been reported among 29 patients (43%) in this study. Overall, 23 patients (49%) reported ≥ 1 SAE during the Dose Escalation phase and 6 patients (30%) reported ≥ 1 SAEs during the Expansion phase. The most frequently reported SAEs overall were pneumonia (6 patients), vomiting (2 patients, plus hematemesis in 1 patient), small intestinal obstruction (3 patients), and stomatitis, esophageal carcinoma, sepsis, and failure to thrive in 2 patients each. SAEs reported in most patients (85%) were considered not study

drug-related, including all of the fatal events. No SAE event terms were reported in >1 patient in the Dose Escalation phase.

Treatment-Emergent Adverse Events

All patients treated in Study INK128-003 reported at least 1 TEAE. The most common (≥ 10% of patients) TEAEs, regardless of causality, that were reported as of the clinical database cutoff include fatigue, nausea, and diarrhea, which were reported in 67%, 60%, and 52% of patients, respectively.

Regardless of causality, TEAEs reported in 54 patients (81%) overall were assessed as severity ≥ Grade 3. The most commonly reported ≥ Grade 3 TEAEs included neutropenia (21% of patients), hypophosphatemia (15%), diarrhea or hyperglycemia (12% of patients each), and fatigue, hypokalemia, and vomiting (10% of patients each).

Events Leading to Study Discontinuation

All but 1 patient had discontinued from TAK-228 treatment in Study INK128-003 as of the clinical data cutoff. Reasons for discontinuation for the other 66 patients included disease progression (54%), patient decision (24%), or ≥ 1 TEAE (21%). Events reported as leading to study discontinuation for more than 1 patient included fatigue (4 patients) and pneumonia, rash (erythematous or macular), failure to thrive, or vomiting (2 patients, each). A majority (52%) of the events were considered not related to TAK-228. A total of 9 events (43%) were considered serious and 12 were assessed as severity ≥ Grade 3, including 3 fatal events. Ten events remained ongoing as of the last contact with the patients.

3.2.4. Study C31001

Study C31001 is a phase 1b/2 study of the safety and efficacy of TAK-228 in combination with exemestane or fulvestrant in women with ER+/HER2– advanced or metastatic breast cancer that has progressed on prior treatment with everolimus in combination with exemestane or fulvestrant. Patients in this study continue receiving their same prior therapy (either exemestane or fulvestrant) at the same dose, in combination with TAK-228.
As of the clinical data cutoff date, 16 patients had received ≥ 1 TAK-228 dose along with either exemestane (7 patients) or fulvestrant (9 patients). A total of 88% of the women treated as of the data cut were white. At baseline, their median age was 56.5 years (range 42-74 years). Of the original 16 patients, 12 remained ongoing as of data cutoff.

Deaths

As of the clinical data cutoff date, no patient had died within 30 days of administration of their last dose of study drug.

Serious Adverse Events

As of the clinical data cutoff date, 3 treatment-emergent SAEs (ataxia, pneumonitis, and upper respiratory tract infection) had been reported in 3 patients (19%). The SAE of ataxia resulted in a dose delay, and no action was taken in response to the other events. All 3 events were reported as

being severity Grade 3 and all had resolved as of the data cut. Only the event of pneumonitis was considered related to study drug.

Treatment-Emergent Adverse Events

The most common (≥ 12% of patients) TEAEs, regardless of causality, include nausea, fatigue, and diarrhea or stomatitis, which were reported in 69%, 50%, and 44% of patients, respectively.

Regardless of causality, the most common TEAEs considered severity ≥Grade 3 were alanine aminotransferase increased, diarrhea, fatigue, and nausea, each of which were reported in 2 patients

Events Leading to Study Discontinuation

Four patients had discontinued from TAK-228 treatment as of the clinical data cutoff. Reasons for discontinuation were disease progression (2 patients), patient decision (1), and ≥ 1 TEAEs (1). The TEAE leading to discontinuation was Grade 3 nausea in a patient in the MLN0218+fulvestrant arm. The event was not considered related to study drug and had resolved as of data cutoff.

3.2.5. Study C31002

Study C31002 is a phase 1 open label, single-arm, multicenter study to evaluate the effect of a single dose of 40 mg TAK-228 on the QT/QTc (QT interval corrected for heart rate) in patients with advanced solid tumors. After completing the per-protocol PK/ECG assessments on Cycle 1, Day 3, patients may continue to receive TAK-228 if, in the opinion of the investigator, the patient is deriving clinical benefit, until they experience disease progression. Patients continuing treatment receive TAK-228 30 mg QW in 28-day cycles.
As of the clinical data cutoff date, 19 patients had received ≥ 1 TAK-228 dose in this study and 3 had entered Cycle 2. A total of 53% are women and 74% are white. At baseline, their median age was 63.5 years (range, 46-76 years). Of the original 19 patients, 16 remained ongoing as of data cutoff.

Deaths

As of data cutoff, no reports of events having fatal outcomes had been reported to the clinical database as of data cutoff.

Serious Adverse Events

Serious adverse event information had not been reported to the clinical database as of the data cutoff date.

Treatment-Emergent Adverse Events

The most common (≥ 10% of patients) TEAEs, regardless of causality, include nausea, fatigue, decreased appetite, and vomiting, which were reported in 53%, 42%, 32%, and 21% of patients, respectively. Information regarding severity of TEAEs had not been reported to the clinical

database as of data cutoff.

Events Leading to Study Discontinuation

As of data cutoff, 2 patients had discontinued due to ≥ 1 AE. The preferred term for 1 event was pelvic pain. The other event had not been coded as of data cutoff; Both events were reported as being Grade 4 in severity, had not yet resolved as of data cutoff, and were not considered study drug-related.

4.0 Updated Manufacturing Process

A new TAK-228 capsule containing milled active pharmaceutical ingredient (API) is available for new clinical studies in 1 mg, 3 mg and 5 mg strengths.

RP2D for milled TAK-228 daily schedule was determined to be 3 mg QD

The selected dose of 3 mg TAK-228 QD is based on the findings from 2 studies: Study INK128-001 and Study MLN0128-1004.

Study INK128-001was the first-in-human study of TAK-228. This was an open-label study designed to determine the maximum tolerated dose (MTD) and to identify dose-limiting toxicities (DLTs) for oral administration of single-agent unmilled TAK-228, and to characterize the safety and tolerability of escalating doses of TAK-228 in patients with advanced solid tumors. In this study, 116 patients with advanced solid tumors received TAK-228 (2 – 40 mg via 4 dosing schedules: QD [once a day] (31 patients), QDx3 QW [3 days per week] (33 patients), QDx5 QW [5 days per week] (22 patients), and QW [once a week] (30 patients) in the dose escalation phase. The MTD of TAK-228 QD was 6mg.
Based on the safety profile, PK/pharmacodynamics, and preliminary efficacy the 5 mg QD and 40 mg QW dosing regimens were selected for further evaluation in the expansion phase in patients with renal cell carcinoma, endometrial cancer, or bladder cancer (n=82).
Scale-up manufacturing of TAK-228 required the introduction of a physical milling step during the granulation process to control for particle size distribution of TAK-228 drug substance. In order to observe whether this milling step altered the safety and PK profile of TAK-228, the recommended dose of milled TAK-228 QD was re-evaluated in Study MLN0128-1004 (see Table below). A total of 17 patients were enrolled and assigned, sequentially to 2 QD dosing cohorts. PK, safety, and tolerability were assessed.

Dose-Limiting Toxicity Observed with once daily TAK-228 in Study MLN0128-1004

Dose of Milled TAK-228	Number of Evaluable Patients	Patients with DLTs observed in Cycle 1
4 mg QD	6	3 (rash, apetite loss and fatigue)
3mg QD	11	1 (decreased platelets)

DLT = dose-limiting toxicity; QD = once daily.

As only 1 patient in the 3 mg QD dose cohort experienced DLT in Cycle 1, the 3 mg TAK-228 QD was determined as MTD for milled TAK-228.

5.0 Clinical Summary of Safety

5.1. Special Warnings and Precautions for Use

5.1.1. Insulin and Glucose Levels

Hyperglycemia and hyperinsulinemia are known toxicities associated with inhibition of mTOR and related pathways based on clinical and nonclinical studies.

A rise in fasting plasma glucose has been observed as early as 1 to 2 days following oral administration of TAK-228. Daily in-home glucose monitoring and early initiation of treatment of the hyperglycemia are essential. Daily home monitoring and early treatment, have resulted in good control of glucose levels for themajority of TAK-228-treated subjects who developed hyperglycemia.

5.1.2. Cardiac Effects

Cardiac events (including QT interval corrected for heart rate prolongation and arrhythmias) have been infrequently observed in clinical studies of 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 related to TAK-228. Routine cardiac monitoring with baseline electrocardiogram (ECG) and on-study ECGs and physical examination constitute the core cardiac safety monitoring in all TAK-228 studies.

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

5.1.3. Renal Function

Elevations in creatinine (regardless of causality) have been observed in subjects receiving TAK-228, all of which have been reversible with drug interruption and/or supportive care with 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 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 4.0, to further evaluate possible etiologies for the renal dysfunction.

5.1.4. Rash

Rash observed in clinical studies of 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.

5.1.5. Pneumonitis

Pneumonitis is a known potential risk of mTOR inhibitors. Early recognition, prompt intervention, and a conservative risk management approach are recommended due to pneumonitis that has been observed with rapalog therapy and with TAK-228 administration. Symptoms of pneumonitis will be closely monitored in all TAK-228 study subjects.

5.2. Interactions With Other Medications and Other Forms of Interaction

Clinical drug-drug interaction studies have not been conducted with TAK-228. At this time, there are no known drug interactions. In vitro data, including cytochrome P450 induction/inhibition and transporter inhibition studies conducted for TAK-228, suggest a low risk for TAK-228 to precipitate a drug-drug interaction. Although potential drug-drug interactions with TAK-228 cannot be ruled out based on the known metabolism characteristics of TAK-228, the potential risk is considered low.

6.0 Study Rationale

6.1. Rationale for trial and selected study population

mTOR is a serine/threonine protein kinase that is dysregulated in many sarcoma subtypes. It regulates metabolism, homeostasis, survival and proliferation. Previous work on complex genomic sarcomas such as LMS, undifferentiated pleomorphic sarcoma (UPS) and MPNST have reported rates of PTEN protein loss in by immunohistochemistry (IHC) or western blot analysis in 29%-68% of the sarcomas.[6,9] Subsequent activiation of AKT has been noted in over 50% of sarcomas with partial or complete loss of PTEN expression.[9] Additionally, in a murine model of LMS, Hernando and collegues have shown the critical role of the AKT/mTORC1 pathway in sarcomagenesis.[4] Similar models in MPNST suggest PTEN dosage and its signaling pathways are critical in transformation from a neurofibroma to MPNST.[18,19] Given the overall poor survival in patients with advanced STS, few new active agents in clinical use, and the importance of the mTOR pathway in this disease, there is rationale for this study.

6.2. Rationale for assays

All potential patients must consent to assessment of a baseline tumor sample prior to entry. This may require a biopsy. This tumor sample will be used to assess PTEN protein and genomic levels in order to determine eligibility. PTEN protein loss will be determined by IHC. For complex genomic sarcomas such as LMS, UPS and pleomorphic rhabomyosarcoma or

pleomorphic liposarcoma, PTEN protein loss appears to occur as a result of deletion of the 10q chromosomal region containing the PTEN locus. Gibault and colleagues showed that partial or complete loss of PTEN gene by array-comparative genomic hybridization (a-CGH) occurs in 39-46% of these sarcomas with complex genomics, and that a partial loss may be sufficient for a pathologic phenotype.[9]

On the other hand, in angiosarcomas and MPNST activation of the PIK3CA/AKT/mTOR pathway is not related to intragenic deletion of PTEN.[7] Promoter hypermethylation of the PTEN gene may play a role in the pathogeneis of this MPNST.[6] PIK3CA mutations found in 14-18% of myxoid and round cell liposarcoma are rare events in other sarcomas.[20,21] Our report of multiplatform molecular profiling in over 2000 sarcomas confirmed PTEN protein loss in approximately 40% of complex genomic sarcomas, with very low rates of PIK3CA and PTEN mutations.[22]

Thereofore, PTEN loss appears to be a relatively frequent event in complex genomic sarcomas, resulting in activation of AKT and the mTOR pathway. Though more commonly this is due to loss of the chromosome 10 region encompassing the PTEN gene, there may be other alterations as well. Thus, PTEN will be assessed at baseline both by protein expression and using SNP-based chromosome microarray analysis. The latter technique will be used to assess PTEN deletion and PIK3CA and PTEN mutations.

6.3. Rational for selected agent

Despite promising preclinical data first-generation inhibitors of mTOR have been unsuccessful clinically. These agents selectively inhibit mTORC1 leaving mTORC2 unchecked, with resultant upstream upregulation of AKT. RICTOR a major component on mTORC2 has been found to be overexpressed in some sarcomas.[9] TAK-228 is a selective competitor of both mTORC1 and mTORC2. In vitro testing of TAK-228 has demonstrated anti-proliferative activity in cell lines of MPNST, synovial sarcoma, rhabdomyosarcoma, liposarcoma and Ewing's sarcoma. This anti-proliferative activity is higher than seen with rapamycin. Similar to rapamycin treatment, TAK-228 resulted in inhibition of phosphorylation of S6K1 and S6 consistent with inhibition of mTORC1 activity. In contrast, TAK-228 also inhibited phosphorylation of NDRG1 at Thr346, suggesting mTORC2 inhibition in sarcoma cell lines. Through western immunoblotting it was also noted that TAK-228 prevented feedback upregulation of pAKT. Treatment with rapamycin on the other hand, led to an increase in pAKT473. Xenograft models of Ewing's sarcoma and MPNST exhibited tumor growth suppression when exposed to TAK-228 and induction of apoptosis was seen in rhabdomyhosarcoma models. Therefore, TAK-228 a selective, highly potent inhibitor of both mTORC1 and mTORC2 appears to be the optimal agent to test in patients with complex genomic sarcomas whose tumors have mTOR pathway dysregulation.

6.4. Rationale for endpoints

Unlike traditional chemotherapeutic agents, non-cytoreductive therapies are not expected to necessarily demonstrate tumor shrinkage, but rather stabilization of disease. In that regard, the European Organization for Research and Treatment of Cancer (EORTC) has identified criteria for success in phase II STS studies using absence of progression (or progression free rate PFR)

as a primary endpoint. References for drug activity are defined as a 6 month PFR ≥ 30-56% depending on histology for first line treatment and a 3 month PFR ≥ 40% in the second line setting.[23] An ineffective agent will have a PFR of ≤ 20% in the second line setting.

7.0 STUDY OBJECTIVES

7.1. Primary Objectives

1. To determine the efficacy of TAK-228 in sarcomas with PI3K/AKT/mTOR pathway dysregulation as measured by progression free rate at 12 weeks using RECIST 1.1.

7.2. Secondary Objectives

1. To determine the safety and tolerability of TAK-228 in this patient population.
2. To determine the objective response rate (ORR) of TAK-228 in this patient population.
3. To determine progression free survival (PFS) and overall survival (OS) in this patient population.

7.3. Tertiary Objectives

1. To determine concordance of PTEN protein loss by IHC and PTEN genomic loss by SNP-based chromosome microarray analysis (CMA).

8.0 STUDY ENDPOINTS

8.1. Primary Endpoints

The primary endpoints include: Progression free rate at 12 weeks

8.2. Secondary Endpoints

The secondary endpoints include: Toxicity per CTCAE v4.03, objective response rate, progression free survival, overall survival

8.3. Tertiary/Exploratory Endpoints

The exploratory endpoints include: Concordance of PTEN protein and genomic loss

9.0 STUDY DESIGN

9.1. Overview of Study Design

This is an open-label phase II study of TAK-228 for patients ≥ 18 years of age with complex genomic sarcomas exhibiting PI3-kinase pathway dysregulation. Patients must have surgically unresectable or metastatic disease that is refractory to at least one prior line of therapy (not including neoadjuvant or adjuvant therapy in a curative setting). Patients disease must also have evidence of progression prior to enrollment. The purpose of this study is to determine the

antitumor activity in this group of patients. Patients must meet all eligibility criteria as detailed in section 10. A total of up to 24 patients will be included in the study. Patients will undergo screening evaluations to determine eligibility within 28 days of the first dose. All patients will be required to submit baseline tumor samples for analysis. Patients who have had their tumors tested commercially for PI3K/AKT/mTOR alterations will be assessed on a case by case basis for eligibility and for determination as to whether additional tissue is required.

TAK-228 will be administered orally at 3 mg daily for a 21 day cycle. Clinical and laboratory assessments will be made on day 1 of each cycle. Disease will be assessed by comparing unidimensional tumor measurements on pre and peritreatment imaging (CT or MRI) after weeks 6, 12, 18 and every 12 weeks thereafter. Response will be assessed according to RECIST 1.1. Therapy will continue until disease progression or unacceptable toxicity or withdrawal of consent.

9.2. Trial Procedures

The study calendar summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

9.2.1. Administrative Procedures

9.2.1.1. Informed Consent

The IRB approved informed consent documents must be signed by the patient, or the patient's legally authorized representative, before his or her participation in the study. The case history for each patient shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent documents must be provided to the patient or the patient's legally authorized representative. If applicable, they will be provided in a certified translation of the local language.

Original signed consent forms must be filed in each patient's study file or medical record with a copy in the study file.

9.2.1.2. Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

9.2.1.3. Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator.

9.2.1.4. Prior and Concomitant Medications Review

9.2.1.4.1. Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-

specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial.

9.2.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial.

9.2.2. Clinical Procedures/Assessments

9.2.2.1. Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

9.2.2.2. Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam will also be performed every cycle during the treatment phase and at the end of treatment visit.

9.2.2.3. Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Study calendar. Vital signs will include temperature, pulse, respiratory rate,weight and blood pressure. In addition, O2 saturation must be measured by office pulse oximetry during screening and at the beginning of every cycle. Height will be measured at screening only.

9.2.2.4. Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the study calender.

9.2.2.5. Tumor Imaging and Assessment of Disease

Tumor imaging with CT of the chest, abdomen and pelvis. MRI may be used if patient unable to undergo CT per treating physician. The same modality of radiological assessment should be used for baseline and on treatment scans. Scans will be performed at baseline, and at week 6, 12 and 18, thereafter every 12 weeks (± 3 days) while on treatment.

9.2.2.6. Tumor Tissue Collection

All patients must provided sufficicent tissue for analysis to determine eligibility. Patients must consent to providing tumor tissue prior to initiation of therapy if sufficient archival tissue is not available. If a biopsy is required, the treating physician should ensure that this can be done safely. Baseline tumor samples will be collected at the Protocol Support Laboratory at FCCC and

distributed to the labs of Lori Rink for IHC and Jianming Pei for CMA. Patients who have had their tumors tested commercially for dysregulation of the PI3-kinase pathway will be assessed on a case by case basis for eligibility and for determination as to whether additional tissue is required.

Tissue requirements:

Archival: Cell block or 15 unstained slides

Biopsy: At least 3-4, 16-18 gauge core (5 mm X 5 mm) will be required

9.2.3. Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below.

9.2.3.1. Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis, βHCG, Thyroid and Coagulation parameters)

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in the study calendar. Laboratory tests for screening will be performed ≤ 28 days before drug initiation. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

9.2.3.2. Fasting blood glucose

Patients will be monitored by fasting serum glucose (FSG) levels at screening and prior to initiation of each cycle. In addition to monitoring at clinic visits, all subjects will be given a glucometer to monitor their daily pre-dose fasting blood glucose (FBG) levels at home. Subjects will record their pre-dose fasting blood glucose levels in their diary. Subjects will be instructed to notify the study staff immediately with any abnormal readings (ie, ≥ 150 mg/dL) for further instructions on the management of their hyperglycemia. 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 glucose testing may be reduced to once weekly if the sponsor investigator approves.

9.2.3.3. Lipid Panel

Patients will be monitored with fasting lipid panels (including tryglycerides and total cholesterol) at screening and prior to initiation of each cycle.

9.2.3.4. HbA1C

To assess overall glycemic control, HbA1C will be checked at screening, Cycle 1, Day 1 and every 3 months thereafter (± 7 days) while on treatment.

9.2.3.5. EKG

To monitor for cardiac stability, EKG assessment will be performed during screening, at end of treatment and as needed during treatment.

9.2.4. Other Procedures

9.2.4.1. Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit will be performed at the time of discontinuation. Any adverse events, which are present at the time of discontinuation/withdrawal, will be followed in accordance with the safety requirements outlined in Section 14.

9.2.4.2. End of Treatment Visit

The end of treatment visit will be conducted when all toxicities have resolved to Grade 1 or lower (or baseline if condition present on study entry) or is deemed to be irreversible. Subjects should have follow-up CT chest, abdomen and pelvis for disease assessment at the end of therapy if clinically feasible.

9.2.4.3. Follow-up Visits

Upon discontinuation of treatment patients will transition to the follow-up stage of the trial. Patients will be assessed every 3 months for the first 2 years, then every 6 months for 2 years, then annually for survival. Subjects who discontinue treatment for reasons other than disease progression should continue to have radiographic assessments of disease at least every 3 months until documented disease progression

9.3. Patient Registration

Eligible participants will be entered on study centrally by the Fox Chase Cancer Center study monitor or their designee. Participants may be registered from 9:00 am to 4:00 pm EST excluding holidays by emailing the study monitor at: **FCCC.MONITOR@fccc.edu**.

This will be a 2 step registration process:

Step 1 Registration: After all the eligibility criteria under "Step 1 Screening Inclusion criteria" and "Step 1 Screening Exclusion criteria" are confirmed , submit the completed registration form, consent, HIPAA signature pages and eligibility checklist via email to study monitor at: **FCCC.MONITOR@fccc.edu**.. Upon receipt of these documents, the study monitor or their designeewill complete the step 1 registration and assign a sequence number to the participant.

Step 2 Registration: Following the assignment of a sequence number proceed with confirming eligibility criteria under "Step 2 Screening Inclusion criteria" (section 10.3.1). Once eligibility criteria (section 10.3.1) is confirmed submit results via email to the study monitor for confirmation of Step 2 registration. The study monitor or their designeewill notify the site by email once step 2 registration is confirmed.

Participants must begin protocol treatment within 7 days of Step 2 registration confirmation.

Issues that would cause treatment delays must be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant will be recorded as withdrawn from study. The Study Monitor must be notified as soon as possible if a participant does not begin protocol treatment as scheduled. For additional registration questions, please email **FCCC.MONITOR@fccc.edu** or call **(215) 728-5544.**

Exceptions to the current registration policies will not be permitted.

9.4. Number of Patients

The number of patients was calculated using a Minimax two-stage design (P0 = .20, P1 = .40, alpha 0.09, beta 0.2). A total of 24 evaluable patients will be required. The first stage will include 14 patients. If there are at least 3 patients with benefit in the first stage, the second stage will be allowed. At the end of the second stage the treatment will be considered effective if at least 8 patients are progression free at 12 weeks out of 24. Patients will be considered enrolled/registered once eligibility is confirmed.

9.5. Duration of Study

Patients will continue on study until disease progression or prohibitive toxicity. Overall survival will be assessed every 3 months for the first 2 years, every 6 months for the next 2 years, and annually until death. The accrual period is estimated to be 24 months.

9.6. Criteria for Discontinuation

Patients will be removed from the study when any of the criteria applies:

- Disease Progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse events
- Treatment held > 21 consecutive days
- Patient required > 3 dose reductions
- Patient becomes pregnant
- Patient decides to withdraw from the study or
- General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator.

10.0 STUDY POPULATION

This will be a 2 step registration process (for details please refer to section 9.3). For Step 1 registration, the patient must meet all inclusion and exclusion criteria listed under "Step 1 Screening Inclusion Criteria" and "Step 2 Screening Exclusion criteria" . For the final Step 2 registration the patient must meet the inclusion criteria listed under "Step 2 Screening Inclusion Criteria" to be enrolled in the study:

10.1 Step 1 Screening Inclusion Criteria

10.1.1. Male or female patients 18 years or older.

10.1.2. Patients must have a diagnosis of a locally advanced or metastatic sarcoma that is progressing. The following subtypes (considered genomically complex) will be eligible: leiomyosarcoma (well differentiated or poorly differentiated), undifferentiated pleomorphic sarcoma, myxofibrosarcoma, pleomorphic rhabdomyosarcoma, pleomorphic liposarcoma, malignant peripheral nerve sheath tumor, angiosarcoma or extraskeletal osteosarcoma. Other potentially genomically complex STS subtypes may be included on a case-by-case basis after discussion with the principal investigator.

10.1.3. Measurable disease by RECIST 1.1 criteria (at least one target lesion outside of previous radiation fields or progressed within a previous radiation field), described in detail in section 15.

10.1.4. Progression of disease by radiographic imaging (10% increase in size by RECIST v1.1 within 6 months of registration) or presence of new lesions.

10.1.5. Must have received at least 1 prior systemic therapy for advanced disease (does not include adjuvant/neoadjuvant therapy in a curative setting).

10.1.6. Eastern Cooperative Oncology Group (ECOG) performance status 0-2.

10.1.7. Adequate contraception as follows:
For women:

- Postmenopausal for at least 1 year before the screening visit, OR
- Surgically sterile, OR
- If they are of childbearing potential, agree to practice 1 effective method of contraception, and 1 additional (barrier) method, at the same time, from the time of signing the informed consent through 90 days (or longer, as mandated by local labeling [eg. 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 (Periodic abstinence [e.g, calendar, ovulation, symptothermal, postovulation methods] and withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception. Female and male condoms should not be used together.)

Highly effective methods	Other effective methods (barrier methods)
Intra-uterine devices (IUD)	Latex condom
Hormonal (birth control pills/oral contraceptives, injectable contraceptives, contraceptive patches, or contraceptive implants)	Diaphragm with spermicide; Cervical cap;Sponge

For men, even if surgically sterilized (ie, status post-vasectomy), they 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 (Periodic abstinence [e.g, calendar, ovulation,

symptothermal, postovulation methods for the female partner] and withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception. Female and male condoms should not be used together.)

- Agree not to donate sperm during the course of this study or 120 days after receiving their last dose of study drug

10.1.8. Screening clinical laboratory values as specified below:

a) Bone marrow reserve consistent with:
   i. absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L;
   ii. platelet count ≥ 100 x 10^9/L;
   iii. hemoglobin ≥ 9 g/dL without transfusion within 1 week preceding study drug administration

b) Hepatic:
   i. total bilirubin ≤ 1.5 x upper limit of normal (ULN),
   ii. transaminases (aspartate aminotransferase/serum glutamic oxaloacetic transaminase-AST/SGOT and alanine aminotransferase/serum glutamic pyruvic transaminase-ALT/SGPT) ≤ 2.5 x ULN (≤ 5 x ULN if liver metastases are present);

c) Renal: creatinine clearance ≥ 50 mL/min based either on Cockroft-Gault estimate or based on urine collection (12 or 24 hour);

d) Metabolic:
   i. Glycosylated hemoglobin (HbA1c) ≤ 7.0%,
   ii. fasting serum glucose ≤ 130 mg/dL
   iii. fasting triglycerides ≤ 300 mg/dL

10.1.9. Ability to swallow oral medications.

10.1.10. Voluntary written consent must be given before performance of any study related procedure not part of standard medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care.

10.1.11. Patients who have a history of brain metastasis are eligible for the study provided that all the following criteria are met:

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

10.2. Step 1 Screening Exclusion Criteria

Patients meeting any of the following exclusion criteria are not to be enrolled in the study:

10.2.1. Any clinically significant co-morbidities, such as uncontrolled pulmonary disease, active central nervous system disease, active infection, or any other condition that could compromise the patient's participation in the study.

10.2.2. Known human immunodeficiency virus infection.

10.2.3. Known hepatitis B surface antigen-positive, or known or suspected active hepatitis C infection.

10.2.4. Any serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with the completion of treatment according to this protocol.

10.2.5. Diagnosed or treated for another malignancy within 2 years before administration of the first dose of study drug, or previously diagnosed with another malignancy and have any evidence of residual disease. Patients with nonmelanoma skin cancer or carcinoma in situ of any type are not excluded if they have undergone complete resection.

10.2.6. Breast feeding or pregnant.

10.2.7. Manifestations of malabsorption due to prior gastrointestinal (GI) surgery, GI disease, or for an unknown reason that may alter the absorption of TAK-228. In addition, patients with enteric stomata are also excluded.

10.2.8. Treatment with any investigational products, radiation therapy, surgery, tumor embolization, chemotherapy or immunotherapy within 21 days before the first dose of the study drug. For biologic or hormonal therapy treatment within 14 days or five half-lives of a drug (whichever is longer) before the first dose of study drug.

10.2.9. History of any of the following within the last 6 months before administration of the first dose of the drug:

- Ischemic myocardial event, including angina requiring therapy and artery revascularization procedures
- Ischemic cerebrovascular event, including transient ischemic attack and artery revascularization procedures
- Requirement for inotropic support (excluding digoxin) or serious (uncontrolled) cardiac arrhythmia (including atrial flutter/fibrillation, ventricular fibrillation or ventricular tachycardia)
- Placement of a pacemaker for control of rhythm
- New York Heart Association (NYHA) Class III or IV heart failure (See Appendix A)
- Pulmonary embolism

10.2.10. Significant active cardiovascular or pulmonary disease including:

- Uncontrolled hypertension (i.e., systolic blood pressure >180 mm Hg, diastolic blood pressure > 95 mm Hg). Use of anti-hypertensive agents to control hypertension before Cycle1 Day 1 is allowed.
- Pulmonary hypertension
- Uncontrolled asthma or O_2 saturation < 90% by arterial blood gas analysis or pulse oximetry on room air
- Significant valvular disease; severe regurgitation or stenosis by imaging independent of symptom control with medical intervention, or history of valve replacement
- Medically significant (symptomatic) bradycardia
- History of arrhythmia requiring an implantable cardiac defibrillator
- 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)

10.2.11. Poorly controlled diabetes mellitus defined as glycosylated hemoglobin (HbA1c) > 7% ; patients with a history of transient glucose intolerance due to corticosteroid administration or gestational diabetes may be enrolled in this study if all other inclusion/exclusion criteria are met.

10.2.12.

10.2.13. Patients receiving systemic corticosteroids (either IV or oral steroids, excluding inhalers or low-dose hormone replacement therapy) within 1 week before administration of the first dose of study drug.

10.2.14. Patients who are taking proton pump inhibitor (PPI) within 7 days before receiving the first dose of study drug or who require treatment with PPIs throughout the trial or those who are taking H2 receptor antagonists within 24 hours of the first dose of study drug.

10.3. Step 2 Screening Inclusion Criteria

10.3.1. Tumor must have dysregulation of the PI3K/AKT/mTOR pathway. For the purposes of this study, patients must have either PTEN protein or genomic loss, or PIK3CA/PTEN mutation. See section 12. Patients must be willing to provide sufficient archival tissue. If this is not available fresh tumor for biopsy is required. In the event that a patient has had tumor analyzed for PTEN/PIK3CA status through commercial means, their eligibility and need for additional tissue will be determined on a case by case basis by the principle investigator.

10.4. Inclusion of Women and Minorities

Men and women, regardless of race, ethnic group or sexual orientation are eligible for this study.

10.5. Pregnancy

It is not known what effects TAK-228 has on human pregnancy or development of the embryo or fetus. Therefore, women participating in this study should avoid becoming pregnant, and men should avoid impregnating a female partner or donating sperm. Women of childbearing potential and men should use effective methods of contraception during and through 90 days after the last dose of study drug, as specified below.

Women must meet 1 of the following:

- Postmenopausal for at least 1 year before the screening visit, OR
- Surgically sterile, OR
- If they are of childbearing potential, agree to practice 1 effective method of contraception and 1 additional effective (barrier) method at the same time from the time of signing of the informed consent form (ICF) through 90 days after the last dose of study drug (or longer, as mandated by local labeling [eg. USPI, SmPC, etc;]

Highly effective methods	Other effective methods (barrier methods)
Intra-uterine devices (IUD)	Latex condom
Hormonal (birth control pills/oral contraceptives, injectable contraceptives, contraceptive patches, or contraceptive implants)	Diaphragm with spermicide; Cervical cap;Sponge

OR

- Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the patient. (Periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods] and withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception.)
- Female and male condoms should not be used together

Men, even if surgically sterilized (ie, status postvasectomy) must agree to 1 of the following:

- 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. (Periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods for the female partner] and withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception.)
- Female and male condoms should not be used together
- Agree not to donate sperm during the course of this study or 120 days after receiving their last dose of study drug.

11.0 STUDY DRUG

11.1. Study Drug Administration

All protocol-specific criteria for administration of TAK-228 must be met and documented before drug administration.

TAK-228 will be administered on an empty stomach. Patients should be instructed to refrain from eating and drinking (except for water and prescribed medications) for 2 hours before and 1 hour after each dose.

Patients will be instructed to take their study medication at approximately the same time on each scheduled dosing day and not to take more than the prescribed dose at any time. Patients must swallow the study medication whole and not chew it, open it, or manipulate it in any way before swallowing. If a patient does not take their TAK-228 dose within the time frame specified (+/-

12 h of the dose), then the dose should be skipped and considered a missed dose. Patients will record any missed doses in their diary and resume drug administration at the next scheduled time with the prescribed dosage. Under no circumstance will a patient repeat a dose or double-up doses.

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.

11.1.1. Dose modification Guidelines

In general, TAK-228 will be administered in continuous cycles, which will continue unless the patient has a Grade 3 or greater TAK-228 -related event (adverse events considered to be at least possibly related to study drug). Guidelines for dose interruption and for dose reduction are as follows:

11.1.1.1. Criteria for Dose Interruption During a Cycle

Administration of TAK-228 will be withheld for treatment-related toxicities that are Grade 3 or higher, despite supportive treatment per standard clinical practice.
The following nonhematologic toxicities at least possibly related to TAK-228 will not require dose interruption:

- Grade 3 or higher nausea and/or emesis in the absence of optimal anti-emetic prophylaxis.
- Grade 3 or higher diarrhea that occurs in the absence of optimal supportive therapy.
- Grade 3 fatigue.

11.1.1.2. Criteria for Dose Reduction

In general, TAK-228 administration will be withheld for TAK-228-related toxicities that are ≥ Grade 3 despite supportive treatment per standard clinical practice. If the event resolves to Grade 1 or Grade 2 within 3 weeks of interrupting treatment (except for hyperglycemia – see below), the patient will resume study treatment at a dose reduced by 1 level. If dose modification is required for patients receiving 2 mg QD, then the dosing frequency should be decreased to 5 days per week instead of decreasing the daily dose administered. If TAK-228 dosing is delayed for more than 21 consecutive days for TAK-228-related toxicity despite supportive treatment per standard clinical practice, or more than 3 dose reductions of TAK-228 are required in a patient, stop TAK-228 therapy, discontinue the patient from the study. Patients should be monitored until resolution of all toxicities to ≤ Grade 1 (or baseline if condition present on study entry) or is deemed to be irreversible. The end of treatment (EOT) visit should be performed at that time. Specific guidelines for clinical events of interest are detailed in section 11.2. These detailed recommendations will supersede these general guidelines when considering events of clinical interest.

Table of Dose Adjustments for dosing on a continuous (QD) schedule	
Dose Level	Dose
1	3 mg QD
-1	2 mg QD
-2	2 mg, 5 days on 2 days off
-3	1 mg, 5 days on 2 days off
Level 1 is the starting dose.	

11.2. Management of Clinical Events

A patient who experiences more than one TAK-228 related AE ≥ Grade 3 for the same toxicity should be discontinued from study unless deriving clinical benefit, in which case, they may restart TAK-228 at a reduced dose level after recovery ≤ Grade 1 and with approval of the Sponsor.

11.2.1. Management of Hyperglycemia

On the basis of the clinical experience in TAK-228 trials, most episodes of hyperglycemia observed occurred within the first 60 days after initiation of treatment with TAK-228 and have been either Grade 1 or Grade 2, and have responded quickly to oral metformin. Hyperglycemia has not been dose-limiting since the institution of a standard regimen for early treatment of hyperglycemia.

All patients developing hyperglycemia during the study will have their glucose closely monitored by study staff. The investigator may choose to continue close monitoring of patients who develop Grade 1 hyperglycemia (fasting glucose >ULN ≤160 mg/dL) or, alternatively, consider initiating treatment with an oral hypoglycemic agent, such as metformin. All patients with ≥Grade 2 hyperglycemia (fasting glucose >160 mg/dL) must be treated aggressively with oral hypoglycemic agents and/or insulin as clinically indicated. The investigator should consult an endocrinologist, if needed, to aid in optimizing the patient's hyperglycemia treatment plan.

It is recommended that patients be initially treated with a fast acting insulin sensitizer such as metformin at 500 mg orally QD, and titrate up to a maximum of 1000 mg orally 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 patients. The dose of oral hypoglycemic agents should be adjusted in patients 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 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 fasting blood glucose 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.
Guidance on study drug dose modification for patients with hyperglycemia is provided in the following table:

Table 1 Management of Hyperglycemia

Grade	Description	Treatment	Dose Modification
1	Fasting blood sugar >ULN ≤160 mg/dL	• Continue close monitoring of blood sugar. • Consider initiation of oral hypoglycemic agent.	None
2	>160 to 250 mg/dL	• Initiate oral hypoglycemic agent and/or insulin as clinically indicated	None
≥ 3	>250 mg/dL	• Initiate oral hypoglycemic agent and/or insulin as clinically indicated	Hold TAK-228 until ≤ Grade 2. Resume TAK-228 based on timing of recovery after maximal treatment: • ≤ 1 week: resume TAK-228 at same dose and schedule. • >1 but ≤ 2 weeks: reduce TAK-228 by 1 dose level • >2 weeks: discontinue patient from the study.
Prevention/Prophylaxis:			

Table 1 Management of Hyperglycemia

Grade	Description	Treatment	Dose Modification
• Follow fasting glucose levels during clinic visits. • Monitor home glucometer test results. • Check HbA1c levels every 3 months during therapy. • Recommend life-style modifications, as appropriate (balanced diet, limited alcohol consumption, increased physical activity). • Most episodes of Grade 1 or 2 hyperglycemia respond quickly to oral metformin. Early initiation of therapy at the lowest therapeutic dose 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.			

HbA1c=glycosylated hemoglobin, ULN=upper limit of normal.

11.2.2. Management of Hyperlipidemia

Guidance on study drug dose modification for patients with hyperlipidemia is provided below

Table 2 Management of Hyperlipidemia

Grade	Description	Treatment	Dose Modification
1	Cholesterol >ULN to 300 mg/dL Triglycerides >150 to 300 mg/dL	None	None
2	Cholesterol >300 to 400 mg/dL Triglycerides >300 to 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 TAK-228 until recovery to ≤ Grade 1. Re-initiate TAK-228 at the same dose level
3	Cholesterol >400 to 500 mg/dL Triglycerides >500 to 1000 mg/dL	Same as for Grade 2.	Hold TAK-228 until recovery to ≤ Grade 1, then reinitiate TAK-228 at a dose reduced by 1 level
4	Cholesterol >500 mg/dL Triglycerides >1000 mg/dL	Discontinue from study	Discontinue treatment
Prevention/Prophylaxis: Life-style modifications, as appropriate (balanced diet, limit alcohol consumption, increase physical activity)			

ULN=upper limit of normal.

11.2.3. Management of Oral Mucositis

Guidance on study drug dose modification for patients with oral mucositis

Table 3 Management of Oral Mucositis

Grade	Description	Treatment	Dose Modification
1	Asymptomatic or mild symptoms.	• Nonalcoholic 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 TAK-228 dose if tolerable • Hold only TAK-228 if intolerable until recovery to ≤ Grade 1, then restart at same dose.
3	Severe pain, interfering with oral intake.	• Same as for Grade 2. • Consider intralesional corticosteroids.	• Hold TAK-228 until recovery to ≤ Grade 1, then restart TAK-228 at a dose reduced by 1 level
4	Life-threatening consequences.	• Same as for Grade 2 • Consider intra-lesional corticosteroids	• Stop TAK-228 and discontinue patient from the study

Prevention/Prophylaxis:

- Initiation of a nonalcoholic mouth wash, or 0.9% salt water rinses 4 to 6 times daily is strongly recommended at the 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.

11.2.4. Management of Rash

Guidance on study drug dose modification for patients with rash is provided below

Table 4 Management of Rash

Grade	Description	Treatment	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 or antibiotics.	None

Table 4 Management of Rash

Grade	Description	Treatment	Dose Modification
≥ 3	Macules/papules covering >30% body surface area with or without symptoms.	Consider treatment with topical steroid cream/ointment, oral anti-histamines, oral antibiotics, and/or pulsed steroids.	Hold TAK-228 until ≤ Grade 2 Resume TAK-228 based on timing of recovery: • ≤ 3 weeks: reduce TAK-228 by 1 dose level • >3 weeks: stop TAK-228 and discontinue patient from the study
Patients who develop Grade 4 rash should permanently discontinue study treatment, unless they derive clinical benefit, in which case they may be retreated at a reduced dose level after recover to ≤ Grade 1 severity. 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 4.03, effective date 14 June 2010).			

Prevention/Prophylaxis:

- Rash should be managed aggressively. The investigator should consider consulting a dermatologist or other specialist, if needed.
- A skin biopsy at the site of rash should be considered as soon as possible after the initial episode.

11.2.5. Management of Nausea/Vomiting

Guidance for patients with nausea and/or vomiting is provided in the table below

Table 5 Management of Nausea/Vomiting

Grade	Description	Treatment	Dose Modification
≤ 2	Loss of appetite with or without decreased oral intake; 1 to 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 TAK-228 until ≤Grade 1, then resume TAK-228 without dose modification. If

			experienced for >72 hours despite optimal therapy, hold TAK-228 until ≤ Grade 1, then resume treatment with the dose of TAK-228 reduced by 1 level.
Prevention/Prophylaxis: Prophylactic use of anti-emetic, and antidiarrheal medications are encouraged and may be used before each TAK-228 dosing as needed throughout the study.			
IV=intravenous, IVF=intravenous fluids, TPN=total parental nutrition.			

11.2.6. Management of Cardiac Abnormalities

Management of Patients with QTc Prolongation

Guidance for TAK-228 dose adjustment for patients exhibiting a prolonged QTc interval is provided below.

Table 6 Management of QTc Prolongation

Grade	Description	Treatment	Dose Modification
1, 2	450 msec <QTc <501 msec	Evaluate for other possible causes (eg, electrolyte disturbance, concomitant medication, etc).	None; continue TAK-228 at the same dose and schedule.

Table 6 Management of QTc Prolongation

Grade	Description	Treatment	Dose Modification
3	QTc ≥501 msec on at least 2 separate EKGs	Evaluate for other possible causes (eg, electrolyte disturbance, concomitant medication).(a) Consider a formal consult by a cardiologist; Notify the study doctor; Additional ECGs should be performed weekly until repeated QTc measurements are below Grade 3.	Hold TAK-228 until evaluation complete, then: if there is a plausible explanation for QT prolongation other than TAK-228, may resume study drug at the same dose after QTc measurements fall below Grade 3. If no alternate explanation, study treatment should be discontinued permanently
4	QTc ≥501 msec or >60 ms change from baseline and Torsade de pointes or polymorphic ventricular tachycardia or signs/symptoms of serious arrhythmia	Discontinue study treatment	Discontinue study treatment

ECG=electrocardiogram, IV=intravenous, msec=milliseconds, QTc=QT interval corrected for heart rate.

(a) A list of medications known to prolong QTc can be found at https://www.crediblemeds.org/new-drug-list/

11.2.7. Management of Other Nonhematologic Toxicities (Including Asthenia, Weakness and Fatigue)

Guidance on dose adjustment for patients with other nonhematologic toxicities is provided below

Table 7 Management of Other Nonhematologic Toxicities (Including Asthenia, Weakness, and Fatigue)

Grade	Description	Treatment	Dose Modification
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.	Initiate appropriate medical therapy and monitor.	If tolerable, then no adjustment is required.
2	Moderate; minimal, local or noninvasive intervention indicated.	Initiate appropriate medical therapy and monitor.	• If tolerable, no adjustment required. • If toxicity becomes intolerable, hold TAK-228 until recovery to ≤Grade 1,

Table 7 Management of Other Nonhematologic Toxicities (Including Asthenia, Weakness, and Fatigue)

Grade	Description	Treatment	Dose Modification
			then reinitiate at same dose.
≥ 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated		Hold TAK-228 until recovery to ≤ Grade 1. Reinitiate TAK-228 at dose reduced by 1 level. Patients who develop Grade 4 nonhematological toxicities (with the exception of isolated non-clinically significant laboratory values) should permanently discontinue study treatment, unless they derive clinical benefit, in which case they may be retreated at a reduced dose level after recovery to ≤ Grade 1 severity.

11.2.8. Management of Aspartate Aminotransferase/Alanine Aminotransferase Elevations

Guidance on dose adjustment for patients with AST/ALT elevations is provided below

Table 8 Management of Aspartate Aminotransferase/Alanine Aminotransferase Elevations

Grade	Description	Treatment	Dose Modification
1	>ULN to 3×ULN	None	None
2[1]	Asymptomatic with levels 3 to 5×ULN; >3×ULN with the appearance of worsening fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia.	• Closely monitor LFTs at least weekly or more frequently as indicated. • Assess patient for other causes of transaminitis (eg, past medical history, concomitant medications).	None
3[2]	>5 to 20×ULN; >5×ULN for >2 weeks	Same as for Grade 2.	Hold TAK-228 until ≤Grade 1; Restart TAK-228 at the same dose. Permanently discontinue study treatment if in combination with Grade 2 total bilirubin elevation when alternative causes cannot be identified (ie, Hy's Law);
4	>20×ULN	Same as for Grade 2.	Stop TAK-228 and discontinue patient from the study.

Table 8 Management of Aspartate Aminotransferase/Alanine Aminotransferase Elevations

Grade	Description	Treatment	Dose Modification
			Permanently discontinue study treatment if in combination with Grade 2 total bilirubin elevation when alternative causes cannot be identified (ie, Hy's Law);
Prevention/Prophylaxis: Ensure proper screening of patients for study participation. [1]Gr. 2 ALT/AST at baseline, no additional monitoring necessary if it remains Grade 2. [2]Gr. 2 ALT/AST at baseline, treatment resumes when value returns to Grade 2.			
LFTs=liver function tests, ULN=upper limit of normal.			

11.2.9. Management of Non-infectious Pneumonitis

Guidance for the management of pneumonitis is provided below

Table 9 Management of Non-infectious Pneumonitis

Grade	Description	Treatment	TAK-228 Dose Modification
1	Asymptomatic: Radiographic findings only.	Rule out infection and closely monitor.	None
2	Symptomatic: Not interfering with activities of daily living.	Rule out infection and consider treatment with corticosteroids until symptoms improve to ≤Grade 1.	Interrupt TAK-228 • When symptoms ≤Grade 1, reinitiate TAK-228 at a dose reduced by 1 level. If no recovery within 4 weeks, then discontinue TAK-228.
3	Symptomatic: Interfering with activities of daily living; Requires administration of oxygen.	Rule out infection and consider treatment with corticosteroids until symptoms improve to ≤Grade 1.	Discontinue TAK-228
4	Life-threatening: Ventilatory support indicated.	Rule out infection and consider treatment with corticosteroids.	Discontinue TAK-228.

11.2.10. Management of Renal Toxicity

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. 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 4.0, to further evaluate possible etiologies for the renal dysfunction.

11.3. Excluded Concomitant Medications and Procedures and potential Drug-Drug interactions

The following medications and procedures are prohibited during the study:

- Other investigational agents including mTOR, PI3Kinase and AKT 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 or low-dose hormone replacement therapy), unless necessary for treatment of TAK-228 related AE, ie, rash or for a transient adverse event not related to TAK-228 if clinically indicated e.g. food allergy.
- Anti-epileptic drugs for subjects with treated brain metastasis
- Concomitant administration of any 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. Examples of PPIs include omeprazole, esomeprazole, pantoprazole, lansoprazole, and rabeprazole.
-

11.4. Permitted Concomitant Medications and Procedures

Prophylactic use of anti-emetic, antinausea, and antidiarrheal medications is encouraged, and these may be administered before the first dose and subsequent doses of study drug, as needed throughout the study, and as clinically indicated per standard practice. When selecting an anti-emetic agent, drugs that do not have an effect on the QT interval, such as palonosetron, are preferred.

Histamine H2 receptor antagonists may be allowed, if needed provided that the histamine H2 receptor antagonist is not taken within 12 hours before and within 6 hours after study drug administration. Patients receiving histamine H2 receptor antagonists before enrollment must stop using these medications for at least 24 hours before their first dose of study drug. Examples of histamine H2 receptor antagonists include ranitidine, famotidine, and nizatidine. Cimetidine, a moderate cytochrome P450 (CYP)1A2 inhibitor, is not recommended as first choice H2 receptor antagonist.

Strong CYP1A2 inhibitors and CYP inducers should be administered with caution, at the discretion of the investigator (see Appendix B). Alternative treatments, if available, should be considered.

Other medications considered necessary for the safety and wellbeing of the patient may be administered at the discretion of the investigator.

11.5. Precautions and Restrictions

The use of live vaccines and close contact with those who have received live vaccines should be avoided during treatment with study drug. Examples of live vaccines are: intranasal influenza, measles, mumps, rubella, oral polio, Bacille Calmette-Guerin, yellow fever, varicella, and TY21a typhoid vaccines.

No dietary restrictions will be imposed on study patients other than daily fasting for glucose monitoring.

Patients who show evidence of hyperglycemia during the study will be encouraged to follow a low carbohydrate diet.

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.

11.6. Description of Investigational Agents

TAK-228 will be supplied as capsules for oral administration. The study drug is available in 2 dose strengths, 1 mg and 3 mg, each containing 1 mg and 3 mg of TAK-228, respectively, in addition to the following inactive ingredients: microcrystalline cellulose (solid filler/diluents), magnesium stearate (lubricant), and hard gelatin capsule. The two dose strengths are formulated into size 2 capsules, and each dose strength is differentiated by color, as listed below:

- TAK-228 capsules, 1 mg - white opaque color
- TAK-228 capsules, 3 mg – orange opaque color

11.7. Preparation, Reconstitution, and Dispensation

TAK-228 study drug will be provided in labeled bottles in accordance with all applicable regulations. Materials provided by the sponsor should be dispensed to patients with clear administration instructions from the investigator.

TAK-228 is an anticancer drug and, as with other potentially toxic compounds, caution should be exercised when handling TAK-228 capsules.

11.8. Packaging and Labeling

TAK-228 will be provided by Millennium and will be handled at the investigative site as open-label material. Sites must store according to the labeled conditions.

TAK-228 will be provided in 30-ct, 60-cc high density polyethylene (HDPE) bottles with polypropylene, child-resistant caps and induction seal.

11.9. Storage, Handling, and Accountability

Upon receipt at the investigative site, drug should be stored in the original bottles until use and stored at room temperature from 15°C to 30°C (59°F to 86°F). All temperature excursions will be reported for assessment and authorization for continued use. All investigational supplies must be stored in a secure area with controlled access and will be stored in original packaging. All drug supplies must be used before the retest expiry date.
Because TAK-228 is an investigational agents, it should be handled with due care. In case of contact with broken capsules, raising dust should be avoided during the clean-up operation. The product may be harmful if inhaled, ingested, or absorbed through the skin. Gloves and protective clothing should be worn during the clean-up operation. The area should be ventilated and the spill site washed after material pick-up is complete. The spilled material should be disposed of as hazardous medical waste in compliance with federal, state, and local regulations. In case of contact with the powder (eg, from a broken capsule), the skin should be washed immediately with soap and copious amounts of water for at least 15 minutes. In case of contact with the eyes, copious amounts of water should be used to flush the eyes for at least 15 minutes. Medical personnel should be notified.
Patients will receive instructions for home storage and administration of TAK-228.

Accountability for TAK-228 at all study sites is the responsibility of the sponsor-investigator.

11.10. Study Compliance

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.

12.0 Correlative/Special Studies

Biomarker assessments will be conducted in collaboration with Lori Rink, Ph.D. and Jianming Pei, Ph.D. Patients must consent to providing tumor tissue prior to initiation of therapy if sufficient archival tissue is not available. If a biopsy is required, the treating physician should ensure that this can be done safely. At least 3-4, 16-18 gauge core (5 mm X 5mm) will be required. If additional cores can be obtained safely, these will also be used in the analysis.See Section 9.2.2.6 for details on amounts of tissue required.

In order to assess PTEN status, tissue obtained from biopsy will be paraffin embedded. Expression of PTEN will be examined by IHC In the event that a patient has had tumor analyzed for PTEN status through commercial means, their eligibility and need for additional tissue will be determined on a case by case basis by the sponsor investigator.

PTEN genomic loss at baseline will be assessed using SNP-based CMA with the Affymetrix GeneChip system. Chromosome Microarray Analysis (CMA) using Affymetrix OncoScan assay The OncoScan assay utilizes the Molecular Inversion Probe (MIP) technology to identify

genomic copy number (CN), loss of heterozygosity (LOH) at whole genome coverage (about 220,000 SNPs) with increased resolution in approximately 900 cancer genes while also currently detecting 74 clinically actionable somatic mutations commonly found in nine cancer genes including *PIK3CA* (p.E542Kc.1624G>A, p.E545Kc.1633G>A , p.Q546Kc.1636C>A, p.H1047Lc.3140A>T and p.H1047Rc.3140A>G) and *PTEN* (p.R130*c.388C>T, p.R130Gc.388C>G, p.R130fs*4c.389delG, p.R130Qc.389G>A, p.R159Sc.477G>T, p.R233*c.697C>T , p.P248fs*5c.741_742insA , p.K267fs*9c.800delA). The assay requires ≤ 80 ng of input genomic DNA and can utilize extremely degraded DNA. The OncoScan® assay has been adopted in a number of research and translational laboratories and its utility across a range of tumor types reported.

Recommended OncoScan assay protocol (www.affymetrix.com) is followed. In short, the copy number and somatic mutation MIP probes were added to the sample DNA and allowed to anneal at 58°C overnight (16-18h) after an initial denaturation at 95°C for 5 min. Each sample is then split into two wells and gap fill reaction is performed by adding dATP (A) and dTTP (T) (A/T) in one well and dGTP (G) and dCTP (C) (G/C) to the other well. Uncircularised MIP probes and genomic DNA are digested by using a cocktail of exonucleases, leaving only MIP probes that have been gap filled by the A/T or G/C nucleotides. The circular MIP probes are then linearized using a cleavage enzyme and amplified by PCR. Following a second round of PCR amplification the 120bp amplicons were cleaved into two fragments with the Haeiii enzyme, of which the smaller (44bp) fragment is to be hybridized onto the OncoScan arrays. Samples are then mixed with hybridization buffer and injected into the OncoScan arrays where they are allowed to hybridize for 16-18 h. At the end of the hybridization period, arrays are stained and washed using the GeneChip Fluidics Station 450 and loaded into the GeneChip Scanner 3000 7G (Affymetrix) where array fluorescence intensities are scanned to generate array images (DAT file). Array fluorescence intensity (CEL) files are automatically generated from DAT files by the ffymetrix GeneChip Command Console (AGCC) Software. The copy number, LOH and somatic mutation are analyzed using Affymetrix Chromosome Analysis Console (ChAS) from the generated CEL files.

13.0 STATISTICAL AND QUANTITATIVE ANALYSES

13.1. Determination of Sample Size

The primary objective of this single-arm study is to investigate the Progression Free Rate (CR+PR+SD) at 12 weeks. This trial uses a Simon 2-stage design to test the alternative hypothesis that the Progression Free Rate (PFR) ≥ 0.40 versus the null hypothesis that PFR ≤ 0.20.

The sample size was determined using a Simon 2-stage design (minimax). This study will enroll 14 patients in the first phase. If ≤ 2 patients are progression free at 12 weeks, the trial will be terminated for futility. If > 2 patients are progression free at 12 weeks, the study will continue to the second phase, enrolling an additional 10 patients, for a total sample size of 24 patients. If > 7 patients are progression free at 12 weeks, the treatment will be considered effective. This design has 80% power with 9% type-I error. If the null hypothesis is true, there is a 64.5% probability of early termination, and an expected sample size of 19.5. Note that after the 14 first-stage

patients have been treated, second-stage patients may be enrolled while the remaining first stage-patients are followed for 12 week progression status. Enrollment of second-stage patients will stop if it becomes impossible to satisfy the futility rule of at least 3 patients being progression free at 12 weeks (e.g. if 1 patient still <12 weeks from treatment, but only 3 successes have been observed).

13.2. Populations for Analysis

All patients receiving at least 1 dose of the study drug will be evaluated for safety. Eligible patients who complete first radiologic assessment (week 6) will be considered evaluable for response. These patients will have their response classified according to the definitions stated below (Section 15.4). (Note: Patients who discontinue study therapy for reasons other than consent withdrawal prior to the first radiologic assessment e.g. clinical or radiologic progression, toxicity or death will be counted as failures (progressed disease). For the primary outcome of PRF at 12 weeks, patients without a 12-week assessment will be considered as progressed.)

13.3. Analysis of ORR, PFS, OS

The Objective Response Rate (ORR) defined as CR+PR will be calculated for the patient sample. Progression Free Survival (PFS), defined as the time from initiation of treatment until disease progression, and Overall Survival (OS), defined as the time from initiation of treatment until death from any cause will be calculated. Survival curves will be generated using the methods of Kaplan and Meier, and the median survival times and corresponding 95% confidence intervals will be calculated.

13.4. Demographic and Baseline Characteristics

Patient characteristics including age, race, gender, sarcoma site, prior treatments, histology, and metastatic disease will be tabulated.

13.5. Pharmacokinetics/Pharmacodynamics/Biomarkers

PTEN protein loss and genomic loss will be assessed in patients. Results of IHC and CMA will be dichotomized as loss vs normal. The proportion of concordant samples will be calculated, as will the proportions exhibiting IHC loss/CMA normal, and CMA loss/IHC normal. Note that either IHC loss or CMA loss or PTEN/PIK3CA mutation is required for inclusion in this study.

13.6. Safety Analysis

All adverse events will be tabulated and summarized by grade and type using standard statistics.

The study will halt for excess toxicity if the rate of grade 4 toxicities (despite maximum supportive care) is greater than or equal to 25%. The study will be terminated early if ever 4 of the initial 14 patients have grade 4 or higher toxicity despite maximum supportive care. Similarly, if at any point 6 patients have grade 4 or higher toxicity the study will also be terminated and declared too toxic. Properties of the stopping rule and final decision rule are tabulated below.

Table 10 - Operating characteristics of toxicity stopping rules							
True toxicity rate	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**	**0.40**
P(early stop)	0.04	0.15	0.30	0.48	0.64	0.78	0.88
P(declare toxic)	0.06	0.20	0.42	0.64	0.81	0.92	0.97

14.0 ADVERSE EVENTS

14.1. Definitions

14.1.1. Adverse Events (AE)

AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, treatment or procedure regardless of whether it is considered related to the medical treatment or procedure *(NCI CTEP Guidelines March 28, 2011).*

14.1.2. Serious Adverse Event (SAE)

SAE is an AE that is fatal or life threatening, requires inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours), persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly/ birth defect. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent any of the above outcomes. A "life-threatening" adverse event places the patient at immediate risk of death in the judgment of the investigator or sponsor.

14.1.3. Severity Rating

The investigator will evaluate the severity of each adverse event. NCI Common Terminology Criteria for Adverse Events (CTCAE v.4.0) or study specific toxicity tables provided in the protocol define severity. If not included in CTCAE v.4.0, severity is expressed in numerical grade using the following definitions:

1. Grade 1: Mild-asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2. Grade 2: Moderate-minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL.
3. Grade 3: Severe-severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.
4. Grade 4: Life-threatening consequences; urgent intervention indicated.
5. Grade 5: Death related to AE

14.1.4. Attribution/Relationship to study drug

1. Definite – clearly related
2. Probable – likely related
3. Possible – may be related
4. Unlikely – doubtfully related
5. Unrelated – clearly not related

14.1.5. Expectedness

An Expected Adverse Event is one where the specificity or severity is consistent with the current information available from the resources.

An Unexpected Adverse Event is one where the nature, severity, or frequency of the event is related to participation in the research is not consistent with either:

1. The known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in (a) the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document, and (b) other relevant sources of information, such as product labeling and package inserts: or
2. The expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject(s) predisposing risk factor profile for the adverse event.
   (OHRP Guidance on reviewing unanticipated problems 2007).

14.2. Recording and Reporting Responsibilities

14.2.1. Investigative site recording responsibilities

1. Upon identification of an AE or SAE, the site investigator will utilize the above definitions to properly classify the event. Each category listed above must be recorded for each event.

2. All AEs and SAEs will be recorded in the "AE case report forms" (CRF) and in progress reports with details about the grade and attribution of each episode, action taken with respect to the study drug, and the patient's outcome will be recorded in the CRF. All events will be recorded on case report forms for the duration of the study until they resolve.

3. All SAEs will be recorded on the FDA MedWatch form 3500a. After submitting the initial report it may be necessary to submit follow up reports to the OCR, Sponsor and the FDA should the event require further investigation.

14.2.2. Investigative site reporting responsibilities:

1. The investigator/ site is responsible to report all SAEs that occur on or after the first day of study treatment to the IST Regulatory Specialist within 24 hours of becoming aware of the event. All subsequent SAEs must be reported for up to 30 days after the last treatment.

   Each investigator is responsible to report all AEs/SAEs to their local IRB following guidelines set by that IRB. The FCCC OCR reserves the right to request an event be reported to the IRB at their discretion. Copies of events reviewed by the IRB must be sent email to the IST Regulatory Specialist at **SAE.FCCC@fccc.edu.**

2. If the investigator or IRB feels the event warrants a revision to the informed consent that was not already initiated by the OCR, draft revisions will be made in track changes and submitted to the OCR for consideration. Any consent revisions must receive OCR approval **prior** to submission to the IRB.

3. Any investigator who is in doubt of whether a particular AE needs to be reported is directed to call the Study Monitor for confirmation with the Sponsor Investigator

4. If the results of an investigator or OCR investigation show an adverse event not initially determined to be reportable is so reportable, the investigator will report the event following the above guidelines based on the date the determination is made.

5. Copies of all related correspondence and reporting documents must be submitted to the IST Regulatory Specialist and will be maintained in the trial master file.

Participating sites should report events to:

IST Regulatory Specialist
Fox Chase Cancer Center
Office of Clinical Research
333 Cottman Avenue
Philadelphia, PA 19111
Phone 215-214-1439
SAE.FCCC@fccc.edu

14.2.3. OCR Reporting Responsibilities:

1. Adverse events which meet all of the following criteria must be reported to all participating institutions for IRB submission within 2 weeks of notification of the event.
   i. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent

document; and (b) the characteristics of the subject population being studied;

ii. Possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

iii. Serious (refer to above definition) or otherwise one that suggests that the research places subjects or others at a greater risk of physical or psychological harm than was previously known or recognized.

2. If the adverse event requires modification of the study protocol and informed consent, these changes will be provided to all participating institutions in the form of an amendment from the OCR for each site's IRB of record along with the report of the adverse event.
3. Copies of all related correspondence and reporting documents will be maintained in a centralized regulatory file for this study at OCR.
4. SAEs that are related, unexpected, fatal, or life-threatening are reportable through the Food and Drug Administration (FDA) MedWatch program by telephone or fax no later than 7 calendar days after initial receipt of the information. Further information on the timing of submissions are as directed by FDA guidelines (http://www.fda.gov/medwatch/index.html). Serious, unexpected events that suggest significant clinical risk will be submitted to within 15 calendar days after initial receipt of this information.

Food and Drug Administration:
Telephone 1-800-FDA-1088
Fax 1-800-FDA-0178
http://www.fda.gov/medwatch/report.htm

The OCR will ensure that the SAE reports are sent to Millennium Pharmacovigilance (or designee) from all sites participating in the study from the time of consent up to and including 30 days after administration of the last dost of TAK-228. Any SAE that occurs at any time after completion of TAK-228 treatment or after the designated follow-up period that the sponsor-investigator and/or sub-investigator considers to be related to any study drug must be reported to Millennium Pharmacovigilance (or designee). Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned). All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).Relationship to all study drugs for each SAE will be determined by the investigator or sub-investigator by responding yes or no to the question: Is there a reasonable possibility that the AE is associated with the study drug(s)?

US and Canada
Toll-Free Fax #: 1-800-963-6290
E-mail: takedaoncocases@cognizant.com

14.3. Procedures for Reporting Drug Exposure During Pregnancy and Birth Events

If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately and permanently discontinue study drug. The sponsor-investigator must fax a completed Pregnancy Form to the Millennium Pharmacovigilance or designee immediately. The pregnancy must be followed for the final pregnancy outcome (i.e., delivery, still birth, miscarriage) and Millennium Pharmacovigilance or designee will request this information from the sponsor-investigator.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the sponsor-investigator must also immediately fax a completed Pregnancy Form to the Millennium Pharmacovigilance or designee. Every effort should be made to follow the pregnancy for the final pregnancy outcome.

14.4. Product Complaints

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact Millennium (see below) and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a Millennium Quality representative.

A medication error is a preventable event that involves an identifiable patient and that leads to inappropriate medication use, which may result in patient harm. While overdoses and underdoses constitute medication errors, doses missed inadvertently by a patient do not. Individuals who identify a potential medication error situation should immediately contact Takeda (see below) and report the event.

For Product Complaints, call
1-844-ONC-TKDA (1-844-662-8532)
E-mail: GlobalOncologyMedinfo@takeda.com
Fax: 1-800-881-6092, Hours Mon-Fri, 9am-7pm ET
(US and International)

Product complaints in and of themselves are not AEs. If a product complaint results in an SAE, an SAE form should be completed and sent to Millennium Pharmacovigilance.

15.0 MEASURES OF EFFECT

Response Evaluation Criteria in Solid Tumors (RECIST)

The Response Evaluation Criteria in Solid Tumors (RECIST 1.1) criteria will be used for objective tumor response assessment. Assessments will be performed every 6 weeks. Once protocol treatment has been completed subjects will be assessed every three months or sooner as indicated and judged by treating physicians.

15.1. Definitions

Evaluable for adverse events. All patients will be evaluable for adverse events from the time of their first treatment with TAK-228.

Evaluable for objective response. Eligible patients who complete first radiologic assessment (week 6) will be considered evaluable for response. (Note: Patients who discontinue study therapy for reasons other than consent withdrawal prior to the first radiologic assessment e.g. clinical or radiologic progression, toxicity or death will be counted as failures.

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.

15.2. 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, as ≥10 mm with CT or MRI scan, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). Tumor lesions in a previously irradiated are are considered measurable if they are progressing.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT or MRI 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.

15.3. 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 28 days before the beginning of the treatment.

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

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).

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

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. 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 permitted.

Cytology, Histology 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. Positive cytology may be useful to document clinical progression when other modalities are not feasible.

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.

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.

15.4. Response Criteria

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

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).

16.0 Data and Safety Monitoring Plan

16.1. Monitoring Plan

FCCC OCR will monitor the medical and study records of each participant accrued throughout the course of the study. In addition, the OCR will collect and report data to the Sponsor Investigator who will review these data on a regular basis at a rate dependent on subject accrual. All serious adverse events (SAEs) will be reviewed on a real time basis first by the study site PI and subsequently by the OCR and Sponsor Investigator as applicable.

16.2. Data Safety Monitoring Committee

Interim analysis of toxicity, outcome and ongoing scientific investigations will be performed at least every 3 months by the Fox Chase Cancer Center Data Safety Monitoring Committee

(FCCCDSMC). In this capacity the FCCCDSMC will serve as an advisory committee to the OCR. The FCCCDSMC will review those aspects of this trial that are outlined in the responsibilities section of the Data and Safety Monitoring Plan (DSMP). If the committee decides that changes should be made to this trial, it will make recommendations in writing to the Study Sponsor Investigator, the Associate Director of Clinical Research, and the Protocol Management Executive Committee, which, in turn, have the authority to approve or disapprove these recommendations. These changes will be discussed with the Study Sponsor Investigator before they are implemented. These changes may include early termination of accrual. Other changes might include altering the accrual goals or changing the eligibility criteria for the trial.

17.0 Administrative

This study will be conducted in accordance will local, state and Federal regulations and according to accepted good clinical practice guidelines.

17.1. Data Reporting

The FCCC Study Monitor will request case report forms to be completed within 2 weeks of the protocol visit. Participating sites are responsible to respond to queries prior to the next scheduled monitoring visit.

The OCR staff are responsible for compiling and submitting data to the Sponsor Investigator and statistician on an ongoing basis for monitoring as described in the data safety monitoring plan and reporting to the FCCC Data and Safety Monitoring Committee.

All patient information will be stored in an EDC system accessible only to the study team members for the purpose of entering, reviewing and analyzing data. Any paper records, such as case report files, produced will be stored in a secure location.

The IST Regulatory Specialist is responsible for distributing and tracking review of all IND Action Letters, Safety Reports, study specific Serious Adverse Events.

17.2. Retention of Records

Time points for the retention of records are described in detail in the contract between the grantor and the OCR and passed on to the participating site. Please refer to the study specific terms for specific time points. In all cases the Study Monitor must be notified of any plans to move records to an offsite location prior to doing so.

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18.0 APPENDICES

Appendix A: New York Heart Association 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. Ninth Ed. Boston, MA: Little, Brown & Co; 1994:253-256.[24]

Appendix B: List of Relevant Cytochrome P450 Inhibitors and Inducers

Moderate CYP1A2 Inhibitors	
cimetidine	methoxsalen

Strong CYP1A2 Inhibitors	
fluvoxamine	ciprofloxacin

Clinically Significant Enzyme Inducers		
carbamazepine	rifabutin	St. John's Wort
phenobarbital	rifampin	
phenytoin	rifapentine	

Source: fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm.

Note that these lists are not exhaustive.