OPEN LABEL, PHASE II TRIAL OF NEOADJUVANT TAK-228 PLUS TAMOXIFEN IN PATIENTS WITH ESTROGEN RECEPTOR (ER)-POSITIVE, HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR TYPE 2 (HER2)-NEGATIVE BREAST CANCER.
ANETT.

Indication:	Patients with newly diagnosed ER-positive, HER2-negative breast cancer
Phase:	II
Version:	11
Protocol History	
Original	25 June 2018

Investigator/Authors and Study Center:
Jenny Chang, M.D.
Houston Methodist Cancer Center
6445 Main Street
Houston, Texas
77030
713-441-0681

IND Number: 132898

IRB Number: Pro00016065

This is an investigator-initiated study. The principal investigator Jenny Chang (who may also be referred to as the sponsor-investigator), is conducting the study and acting as the sponsor. Therefore, the legal/ethical obligations of the principal investigator include both those of a sponsor and those of an investigator.

PROTOCOL SUMMARY

Study Title: Open Label, Phase II Trial Of Neoadjuvant TAK-228 Plus Tamoxifen In Patients With Estrogen Receptor (ER)-Positive, Human Epidermal Growth Factor Receptor Type 2 (HER2)-Negative Breast Cancer

Phase: II

Number of Patients: 35

Study Objectives:

Primary

- To evaluate Ki67 before and after treatment with the mammalian target of rapamycin complex (mTORC) 1 and 2 inhibitor TAK-228 plus the non-steroidal agent tamoxifen in patients with newly diagnosed ER-positive, HER2-negative breast cancer.

Secondary

- To evaluate the pathological complete response (pCR) rate after treatment with TAK-228 plus tamoxifen in patients with newly diagnosed ER-positive, HER2-negative breast cancer. pCR will be defined as the absence of residual invasive and in situ cancer on hematoxylin and eosin evaluation of the complete resected breast specimen and all sampled regional lymph nodes following completion of neoadjuvant systemic therapy (i.e., ypT0 ypN0 in the current American Joint Committee on Cancer staging system).
- To evaluate the preoperative endocrine prognostic index score after treatment with TAK-228 plus tamoxifen in patients with newly diagnosed ER-positive, HER2-negative breast cancer.
- To assess the toxicity and safety of TAK-228 plus tamoxifen in patients with newly diagnosed ER-positive, HER2-negative breast cancer.

Exploratory

- To determine the plasma pharmacokinetics of the TAK-228 plus tamoxifen combination.
- To assess the correlation between pCR to TAK-228 plus tamoxifen and changes in Ki67, p53/p63/p73, phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mTOR, and NFkB pathways in ER-positive, HER2-negative breast tumors.
- To assess tumor mutational status to identify predictors of response to the TAK-228 plus tamoxifen combination.
- To evaluate Oncotype DX recurrence score before and after treatment with TAK-228 plus tamoxifen.

Overview of Study Design:

This open label phase II trial is designed to determine the efficacy and safety of TAK-228 plus tamoxifen in patients with newly diagnosed ER-positive, HER2-negative breast cancer. TAK-228 (30 mg weekly [QW]) plus tamoxifen (20 mg daily [QD]) will be orally (p.o.) administered for 16 weeks. The treatment scheme is shown below. Dose adjustments for TAK-228-associated toxicities will be made based on previous literature. Baseline assessment, prespecified visits, and follow-up visit will be conducted as described below.

To evaluate the efficacy of the study treatment, Ki67 will be measured pre- and post-treatment, with the expectation of a post-treatment reduction. Based on prior data for Ki67 changes in the tamoxifen only arm of the IMPACT trial, we will assume null and alternative hypotheses of 60% and 80% reduction in Ki67, respectively. Transformations of the geometric mean and 95% confidence interval will be employed to obtain mean and standard deviation estimates.

Study Population:

Patients with newly diagnosed ER-positive, HER2-negative breast cancer. Patients ineligible for inclusion are those with metastatic disease; those previously treated with hormonal therapy (tamoxifen, aromatase inhibitor) or PI3K, AKT, dual PI3K/mTOR, TORC1/2, or TORC1 inhibitors; and those with known hypersensitivity to

tamoxifen or mTOR inhibitors.
Duration of Study: 16 weeks.

FIGURE 1 STUDY OVERVIEW DIAGRAM

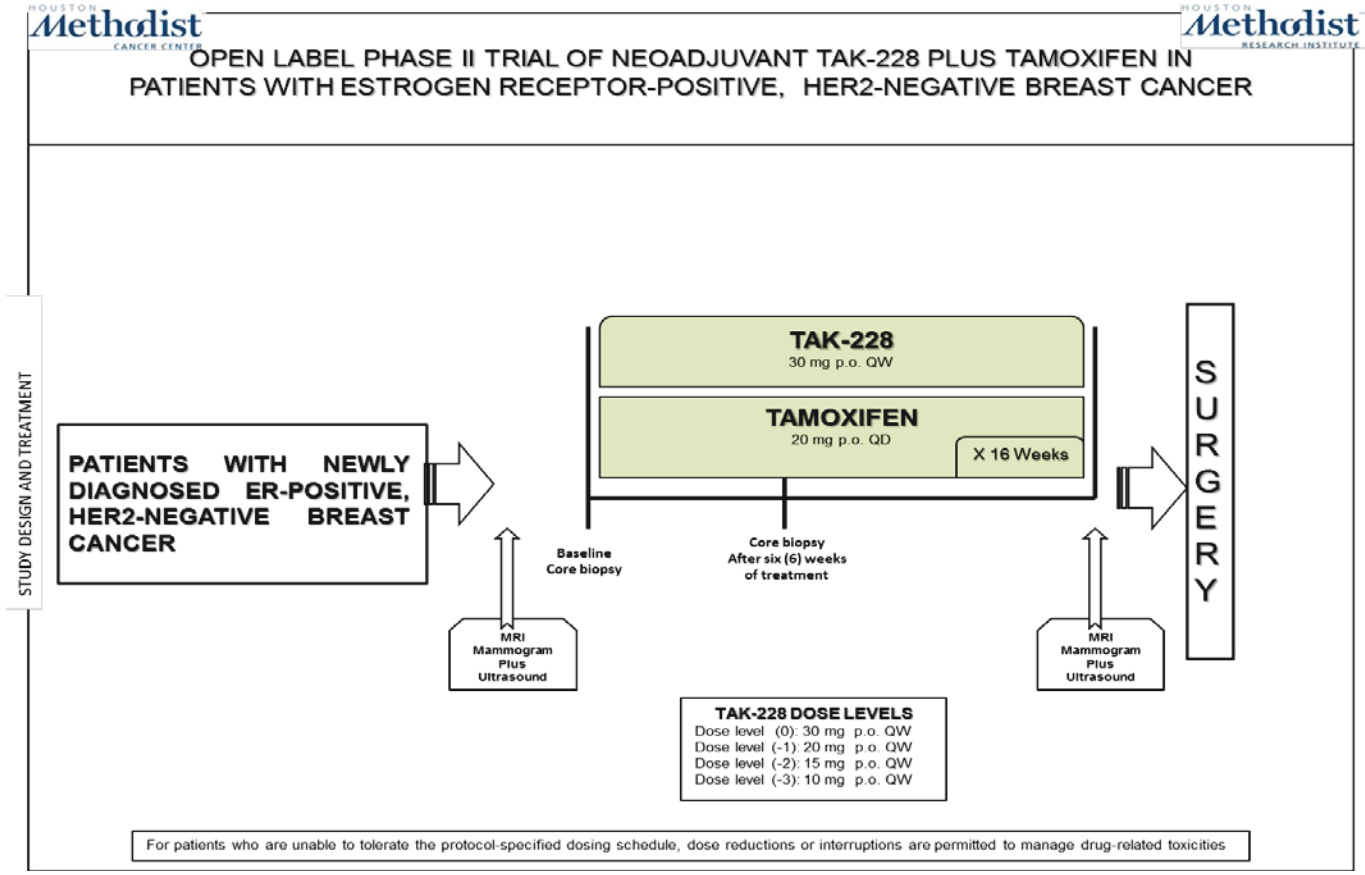


TABLE 1 SCHEDULE OF EVENTS

Visit	Screening[a]	Baseline[b]	V1	V2	V3	V4	V5	V6	V7*	EOT
Week	**-4 to -3**	**-2 to -1**	**1**	**3**	**4**	**6**	**8**	**12**	**16***	
Informed Consent	X									
Inclusion/Exclusion	X									
Demographics	X									
Medical History	X									
Physical Exam[c]		X	X	X	X	X	X	X	X	X
Height	X									
Weight	X	X	X	X	X	X	X	X	X	X
ECOG PS	X	X	X	X	X	X	X	X	X	X
Vital Signs[d]	X	X	X	X	X	X	X	X	X	X
12-Lead ECG[e]		X								X
Hematology[f]		X	X	X	X	X	X	X	X	X
Clinical Chemistry[g]		X	X	X	X	X	X	X	X	X
FSH/LH/Estradiol[h]		X								
Serum Pregnancy (β-hCG)[i]		X								
Urinalysis[j]		X	X	X	X	X	X	X	X	X
Fasting Lipid Panel[k]		X			X		X	X	X	X
Bilateral Mammogram and US[l]		X								X
Breast MRI[m]		X								X
Clinical Breast Exam		X			X		X	X	X	X
Concomitant Therapies		**Continuous from screening period**								
Tumor Assessments (RECIST 1:1)		X								X
Biopsy[n]		X				X				
Tumor Tissue[o]		X				X				
TAK-228 Administration[p]				**Weekly from day 1, week 1 through day 1, week 16**						
Tamoxifen Administration[p]				**Continuous daily from day 1, week 1 through day 7, week 16**						
AEs and SAEs				**From ICF signing up to and including 30 days after local treatment[q]**						
Pharmacokinetics[r]				**Days 1 and 15**						

Abbreviations: AE = adverse event; ALT = alanine transaminase; ANC = absolute neutrophil count; aPTT = activated partial thromboplastin time; ASP = aspartate transaminase; β-hCG = beta-human chorionic gonadotropin; CBC = complete blood count; ECG = electrocardiogram; ECOG PS = Eastern Cooperative Oncology Group performance status (see Appendix A); EOT = end of treatment; FBG = fasting blood glucose; FSH = follicle stimulating hormone; HbA1c = hemoglobin A1c; HDL-C = high-density lipoprotein cholesterol; ICF = informed consent form; INR = international normalized ratio; LDL-C = low-density lipoprotein cholesterol; LH = luteinizing hormone; MRI = magnetic resonance imaging; p.o. = orally; PT = prothrombin time; QD = daily; QW = weekly; RECIST = Response Evaluation Criteria in Solid Tumors; SAE = serious adverse event; SOC = standard of care; US = ultrasound; V = visit; WBC = white blood cell. Tests and procedures should be done on schedule, but V windows of ± 4 days are allowed (except as otherwise specified) occasionally for holidays, vacations, and other administrative reasons or if extenuating circumstances prevent a patient from beginning treatment or completing a scheduled assessment within this time frame. Patients will receive surgery within 30 days of the final dose of study treatment. EOT is defined as 7 to 10 days after the final dose of study treatment.

a. Within 28 days prior to the week 1, day 1 dose of TAK-228 plus tamoxifen.

b. Within 7 days prior to the week 1, day 1 dose of TAK-228 plus tamoxifen. Only screening procedures not performed within 7 days of dosing are required at baseline.

c. The baseline symptom-directed medical history and physical examination are not required if the screening medical history and physical examination were conducted within 3 to 7 days prior to week 1, day 1.

d. Vital sign (blood pressure, heart rate, and oral or temporal temperature) measurements will be obtained during screening, at baseline, and before every V.

e. A 12-lead ECG will be performed at baseline, at EOT, and when clinically indicated.

f. A blood sample for CBC with platelet count and differential WBC count will be obtained at baseline; every V [±1 day]; at EOT; and when clinically indicated. If a patient is found to have an ANC <500/mm^3, platelet count < 25,000/mm^3, or both, the CBC with differential should be repeated at least every other day until the ANC and platelet count have exceeded these values.

g. A blood sample for the clinical chemistry panel (albumin, sodium, potassium, carbon dioxide, chloride, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, AST, and ALT) and evaluation of magnesium, uric acid, and lactate dehydrogenase will be obtained at baseline; every V [±1 day]; at EOT; and when clinically indicated. Fasting serum glucose is to be repeated every week for the length of the study and during follow-up. In-home daily glucose monitoring will be performed by the patients. All patients will be given a glucometer to monitor their daily FBG levels at home. FBG level should be measured after at least 8 hours of fasting. On scheduled TAK-228 dosing days, FBG will be measured before TAK-228 dose. Patients with any abnormal readings (i.e., ≥ 150 mg/dL) will be instructed to notify the study staff immediately for further instructions on the management of their hyperglycemia. Hyperglycemia observed during home glucose monitoring should be confirmed in the clinic. Investigators will be responsible for reviewing the home glucose monitoring logs for hyperglycemia. If no irregularities in the FBG level are observed during a minimum of 2 consecutive months, then the frequency of home fasting 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 FBG levels that exceed 150 mg/dL and, if blood glucose levels are not well-controlled or the patient requires either oral hypoglycemic agents or insulin to control blood glucose levels, then the frequency of home testing of FBG levels will be reinstated to daily. HbA1c level will be measured at screening, week 8, and EOT. Coagulation parameters (PT/INR and aPTT) will be measured at baseline, every V, and EOT.

h. FSH/LH/Estradiol will be measured in all patients at baseline.

i. For women of childbearing potential, the results of a serum β-hCG pregnancy test must be negative within 7 days before the first treatment dose is administered. If the screening serum β-hCG pregnancy test is performed more than 7 days before dosing, it must be repeated at baseline, with results known to be negative prior to administration of the first dose of the study drugs. β-hCG pregnancy testing is to be repeated as clinically indicated.

j. Urinalysis will be performed at baseline, every V, and EOT.

k. A fasting lipid panel will be obtained at baseline; week 4, 8, 12, and 16 of treatment; and EOT. This panel includes total cholesterol, HDL-C, LDL-C, and triglycerides.

l. Bilateral mammogram and US will be performed at baseline and EOT. **NOTE:** Bilateral mammogram and US performed as SOC can be used for screening/baseline if performed within 90 days prior to the week1, day 1 dose of TAK-228 plus tamoxifen.

m. Breast MRI will be performed at baseline and EOT. **NOTE:** Breast MRI performed as SOC can be used for screening/baseline if performed within 60 days prior to the week 1, day 1 dose of TAK-228 plus tamoxifen.

n. In patients with accessible tumor, biopsies will be conducted at baseline and week 6 of treatment, and if the patient progresses while on treatment, before the patient starts the new treatment.

o. Banked tumor tissue obtained as part of the patient's standard care and additional biopsies will be evaluated at a later time to determine the correlation of specific molecular markers (ER; Ki67; mitotic index; apoptosis; levels of S6K, 4EBP-1, EIF4E, EIF4G, and EIF4A; levels of phosphorylated S6K, p53, p63, and p73; and p73 and p63 gene signatures) with clinical parameters such as tumor response. These correlative studies will help define a biomarker signature associated with p63/p73 and/or PI3K/AKT dependence and NFkB in ER-positive breast cancers as well as identify new therapeutic options for this group of patients. Tumor mutational analysis will also be performed to identify predictors of treatment response. Oncotype DX testing will also be performed on biopsy and surgical tissue samples.

p. TAK-228 30 mg p.o. QW for 16 weeks plus tamoxifen 20 mg p.o. QD for 16 weeks. Adjust dose accordingly with Dose Level (see TAK-228 dose modifications).

q. AEs and SAEs will be captured from the time of ICF signing up to and including 30 days after local treatment. Study treatment-related SAEs that occur beyond 30 days after local treatment and any study patient death should also be reported.

r. Blood samples for PK analysis will be obtained 1 hour (± 15 minutes) before TAK-228 dosing and 1 hour (± 5 minutes) after TAK-228 dosing on days 1 and 15. Collect blood samples (10 ml per sampling timepoint) and batch the **plasma** for future analyses.

*Patients will be treated until completion of 16 weeks.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Abbreviation	Term
AE	adverse event
AI	aromatase inhibitor
AKT	protein kinase B
ALT	alanine aminotransferase
ANC	absolute neutrophil count
API	active pharmaceutical ingredient
AST	aspartate aminotransferase
AUC	area under the curve
C_{max}	maximum plasma concentration
CBR	clinical benefit rate
CI	confidence interval
CR	complete response
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
CYP	cytochrome P_{450}
DDI	drug-drug interaction
DLT	dose-limiting toxicity
ECG	electrocardiogram
4EBP-1	eukaryotic translation initiation factor 4E binding protein-1
EIF	eukaryotic translation initation factor
ER	estrogen receptor
EOT	end of treatment
FBG	fasting blood glucose
FDA	Food and Drug Administration
FSG	fasting serum glucose
GI	gastrointestinal
GLP	Good Laboratory Practice
HbA1c	glycosylated hemoglobin
HER2	human epidermal growth factor receptor 2
HR	hazard ratio
IB	investigator brochure

Abbreviation	Term
ICF	informed consent form
IC50	half-maximal inhibitory concentration
IHC	immunohistochemical
IV	intravenous
mBC	metastatic breast cancer
MM	multiple myeloma
MTD	maximum tolerated dose
mTOR	mammalian target of rapamycin
mTORC	mammalian target of rapamycin complex
NCI	National Cancer Institute
NHL	non-Hodgkin Lymphoma
OCT	organic cation transporter
PAX2	paired box 2
pCR	pathological complete response
PD	progressive disease
PEPI	preoperative endocrine prognostic index
PFS	progression-free survival
PI3K	phosphatidylinositol 3-kinase
PK	pharmacokinetics
p.o.	*per os*; by mouth (orally)
PPI	protein pump inhibitor
PR	partial response
QD	once daily
QD×3 days per week	once daily for 3 consecutive days followed by a 4-day dosing holiday every week
QD×5 days per week	once daily for 5 consecutive days followed by a 2-day dosing holiday every week
QTc	heart rate-corrected QT interval
QW	once weekly
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	recommended phase II dose
S6K1	ribosomal protein S6 kinase 1
SAE	serious adverse event
SD	stable disease
SSRI	selective serotonin reuptake inhibitor
tmax	time to reach Cmax

Abbreviation	Term
TEAE	treatment-emergent adverse event
TTP	time to progression
ULN	upper limit of normal
US	ultrasound
WM	Waldenström's macroglobulinemia

1. BACKGROUND AND STUDY RATIONALE

1.1 Scientific Background

STUDY RATIONALE

The two distinct mammalian targets of rapamycin (mTOR) complexes, mTORC1 and mTORC2, play crucial roles in regulating tumor cell growth, metabolism, and motility.[1] Clinical trials with mTOR inhibitors have shown promising results. The open label, phase II TAMRAD (Tamoxifen plus Everolimus) study evaluated the efficacy of the mTOR inhibitor everolimus combined with tamoxifen in endocrine therapy (aromatase inhibitor [AI])-resistant patients. AI-pretreated patients were selected to enrich for phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mTOR-driven tumors, which would be more likely to be sensitive to mTOR inhibition. Postmenopausal women with AI-resistant hormone receptor–positive, human epidermal growth factor receptor 2 (HER2)-negative, metastatic breast cancer (mBC) were randomly assigned to receive tamoxifen 20 mg daily (QD) plus everolimus 10 mg QD (n=54) or tamoxifen alone (n=57). Randomization was stratified by primary and secondary hormone resistance. The primary endpoint was clinical benefit rate (CBR), defined as the percentage of all patients with complete response, partial response, or stable disease at 6 months. The CBR was 61% (95% confidence interval [CI], 47–74) with tamoxifen plus everolimus and 42% (95% CI, 29–56) with tamoxifen alone. Time to progression (TTP) increased from 4.5 months with tamoxifen alone to 8.6 months with tamoxifen plus everolimus, corresponding to a 46% reduction in risk of progression with the combination (hazard ratio [HR], 0.54; 95% CI, 0.36–0.81). Risk of death was reduced by 55% with tamoxifen plus everolimus versus tamoxifen alone (HR, 0.45; 95% CI, 0.24–0.81). The main toxicities associated with tamoxifen plus everolimus were fatigue (72% *vs.* 53% with tamoxifen alone), stomatitis (56% *vs.* 7%), rash (44% *vs.* 7%), anorexia (43% *vs.* 18%), and diarrhea (39% *vs.* 11%). This study suggests that tamoxifen plus an mTOR inhibitor increase CBR, TTP, and overall survival compared with tamoxifen alone in postmenopausal women with AI-resistant mBC.[2]

Results from the BOLERO 2 trial demonstrated that an mTOR inhibitor (everolimus) combined with an AI (exemestane) improves progression-free survival (PFS) in patients with hormone receptor-positive advanced breast cancer previously treated with nonsteroidal AIs. The PFS rate for patients treated with exemestane and everolimus was 7.8 months compared with 3.2 months for those treated with exemestane alone (HR, 0.45, 95% CI, 0.38–0.54, $P < 0.0001$). This treatment combination also proved to be safe. The most common grade 3/4 adverse event (AE) was stomatitis (8% in the everolimus plus exemestane group *vs.* 1% in the placebo plus exemestane group). Here, we propose to determine the efficacy of the mTORC1/2 inhibitor TAK-228 in combination with tamoxifen in patients with newly diagnosed hormone receptor-positive breast cancer by evaluating the change in Ki67 and pathological complete response (pCR) rate.

BACKGROUND

mTOR activity is frequently dysregulated in human cancers. mTORC1 and mTORC2 regulate cell growth, metabolism, angiogenesis, and cell survival and are currently being investigated as potential cancer therapeutic targets. TAK-228 (Millennium Pharmaceuticals) alone and in combination with conventional agents has demonstrated cytotoxic and antiproliferative activity in cells lines with mTORC1 and/or mTORC2 activity.[3] TAK-228 is a potent and selective ATP-dependent mTORC1/2 inhibitor and is structurally and mechanistically distinct from rapamycin and the rapalogs, which only partially inhibit mTORC1.[4] Phase I and II studies of TAK-228 monotherapy and combination therapies are being conducted in patients with solid and liquid malignancies. The purpose of these studies is to determine the highest dose of TAK-228 that can be given safely to patients. Millennium Pharmaceuticals-sponsored clinical trials of TAK-228 in combination with exemestane or fulvestrant in postmenopausal women with

estrogen receptor (ER)/progesterone receptor-positive mBC (NCT02049957) and in combination with the oral PI3Kα inhibitor TAK-117 (previously MLN1117; NCT01899053) are currently ongoing and the results are awaited.[5,6] In a phase I study, Infante et al. determined the maximum tolerated dose (MTD) of TAK-228 to be 6 mg orally (p.o.) QD. Common TAK-228-related toxicities included hyperglycemia and rash, which were manageable and reversible.[1]

Tamoxifen is a non-steroidal agent with potent antiestrogenic properties. Tamoxifen competitively binds to ERs on tumor cells and other tissue targets, producing a nuclear complex that decreases DNA synthesis and inhibits estrogen effects. Tamoxifen causes G_0 and G_1 cell cycle arrest. Tamoxifen is cytostatic rather than cytocidal, as it prevents (pre)cancerous cells from dividing but does not cause cell death. Tamoxifen itself is a prodrug, having relatively little affinity for its target protein, the ER. It is metabolized in the liver by cytochrome P450 (CYP)2D6 and CYP3A4 into active metabolites such as 4-hydroxytamoxifen (afimoxifene) and N-desmethyl-4-hydroxytamoxifen (endoxifen),[7,8] which have a 30–100 times greater affinity for the ER than tamoxifen itself. These active metabolites compete with endogenous estrogen for ER binding. In breast tissue, 4-hydroxytamoxifen acts as an ER antagonist to inhibit the transcription of estrogen-responsive genes.[9] Tamoxifen binds to the ER, which in turn interacts with DNA. The ER/tamoxifen complex recruits other proteins known as corepressors, including NCoR and SMRT, to inhibit estrogen-responsive genes.[10] Tamoxifen function can be regulated by a number of different variables including growth factors.[11] High HER2 expression levels have been shown to induce tamoxifen resistance.[12] Tamoxifen seems to require the protein paired box protein 2 (PAX2) for its full anticancer effect.[10,13,14] In the presence of high PAX2 expression, the tamoxifen/ER complex is able to suppress the expression of the proproliferative HER2 protein. In contrast, when amplified in breast cancer-1 expression is higher than PAX2, the tamoxifen/ER complex upregulates HER2 expression, resulting in the stimulation of breast cancer growth.[10,14]

Tamoxifen is currently used for the treatment of both early and advanced ER-positive breast cancer in premenopausal and postmenopausal women[15] and is the most common hormone treatment for male breast cancer.[16] The Food and Drug Administration (FDA) has approved tamoxifen for the prevention of breast cancer in high-risk women[17] and contralateral breast cancer. In 2006, the large STAR clinical study concluded that the ER antagonist raloxifene is equally effective as tamoxifen in reducing the incidence of breast cancer. Furthermore, after a mean 4-year follow-up, the incidence of uterine cancers and blood clots was 36% and 29% lower in women taking raloxifene than in women taking tamoxifen, respectively, although these differences were not statistically significant.[18-20]

Studies have aimed to identify new therapeutic strategies to overcome endocrine therapy resistance in hormone receptor-positive breast cancers.[21] An emerging mechanism of endocrine resistance is aberrant PI3K/AKT/mTOR signaling.[21-23] Growing evidence supports a close interaction between mTOR and ER signaling pathways. The mTORC1 substrate ribosomal protein S6 kinase 1 (S6K1) phosphorylates activation function domain 1 of the ER, which is responsible for ligand-independent receptor activation.[24,25] Non-steroidal endocrine therapy is the cornerstone of treatment for patients with newly diagnosed and advanced hormone receptor-positive breast cancer. Tamoxifen has become the treatment of choice in premenopausal and postmenopausal patients with newly diagnosed ER-positive breast cancer.[26-30] Unfortunately, not all patients respond to neoadjuvant nonsteroidal endocrine therapy (primary or *de novo* resistance), and even patients who initially respond eventually relapse (acquired resistance). For disease progression, first-line treatment options include AIs (steroidal or nonsteroidal) and the ER antagonists fulvestrant and raloxifene.[31-33]

A minimum percentage of women with ER-positive, HER2-negative breast cancer will respond to

neoadjuvant tamoxifen therapy. Based on clinical findings and other data, ER-targeted therapy combined with a mTORC1/2 inhibitor may achieve high blockade of proliferation pathways and better pCR rates than ER-targeted therapy alone in newly diagnosed ER-positive breast cancer patients. Here, we propose an open label phase II clinical trial to determine the efficacy (change in Ki67, pCR rate, and preoperative endocrine prognostic index score [PEPI] score) and safety of TAK-228 plus tamoxifen in patients with newly diagnosed ER-positive breast cancer. Serial cancer tissue samples will be obtained for molecular studies of tumor response. Toxicity will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 (see Appendix A). Tumor assessment will include mammogram, breast ultrasound (US), and breast magnetic resonance imaging at baseline and end of treatment (EOT). Hematologic function, biochemical measures, vital signs, renal function (urinalysis), and coagulation parameters (prothrombin time/international normalized ratio, activated partial thromboplastin time) will be assessed at baseline, every visit, and EOT. Hemoglobin A1c (glycosylated hemoglobin; HbA1c) will be assessed at baseline, week 8, and EOT. Lipid profile will be assessed at baseline; week 4, 8, 12, and 16 of treatment; and EOT. Tissue will be collected at baseline and week 6 of treatment and from the surgical specimen. Blood samples for pharmacokinetics (PK) analysis will be obtained 1 hour (± 15 minutes) before TAK-228 dosing and 1 hour (± 5 minutes) after TAK-228 dosing on days 1 and 15. Blood samples (10 ml per sampling timepoint) will be collected and **plasma** batched for future analyses.

1.1.1 Disease Under Treatment

Newly diagnosed ER-positive, HER2-negative breast cancer

1.1.2 TAK-228

Millennium has developed TAK-228, a novel, highly selective, orally bioavailable ATP-competitive inhibitor of the serine/threonine kinase mTOR. TAK-228 (formerly INK128) selectively and potently inhibits mTOR (half-maximal inhibitory concentration [IC50] = 1.1 nM), inhibits mTORC1/2 signaling, and blocks cell proliferation. mTOR regulates cell growth, translation, angiogenesis, and cell survival by integrating nutrient and hormonal signals.[34] mTOR plays a key role in several pathways that are frequently dysregulated in human cancer. mTORC1 is best known as a key regulator of protein translation through phosphorylation of eukaryotic translation initiation factor 4E binding protein-1 (4EBP-1) and S6K1. mTORC2 is best known for its ability to fully activate (S473 phosphorylation) AKT, which regulates proliferation and survival pathways.[35]

mTORC is an important therapeutic target as it is a key intracellular point of convergence for a number of cellular signaling pathways. mTOR inhibition may suppress abnormal cell proliferation, tumor angiogenesis, and abnormal cellular metabolism, thus providing the rationale for the use of mTOR inhibitors alone or in combination with chemotherapeutic agents in the treatment of solid and hematological malignancies. Rapamycin and newly approved rapalogs (temsirolimus and everolimus) are specific and allosteric inhibitors of mTORC1. These agents only partially inhibit mTORC1 signaling pathways and do not inhibit mTORC2, which has shown to be an emerging therapeutic target in cancer research. TAK-228, which targets both mTORC1 and mTORC2, was developed to address the incomplete inhibition of the mTOR pathway by rapalogs.
TAK-228 is being developed for both oncology and non-oncology indications. TAK-228 monotherapy and combination therapies with chemotherapy, other molecularly targeted therapies, and antihormonal agents are being investigated for the treatment of advanced solid and hematologic malignancies.

2. NONCLINICAL SUMMARY

2.1 Pharmacology

TAK-228 selectively and potently inhibits mTOR (IC50 = 1.1 nM), inhibits mTORC1/2 signaling, and blocks cell proliferation. TAK-228, administered p.o. in multiple human tumor xenograft mouse models, inhibited angiogenesis and tumor growth by inhibiting mTOR signaling at plasma concentrations associated with *in vitro* inhibition of mTOR in a dose- and time-dependent manner. These effects display a clear PK-to-pharmacodynamic relationship.[3] TAK-228 inhibits both the phosphorylation of S6 and 4EBP1, the downstream substrates of TORC1, and selectively inhibits AKT phosphorylation at Serine 473, as evidenced by decreased phosphorylated AKT, the downstream substrate of TORC2.[3-5] TAK-228 inhibits mTOR signaling and has demonstrated anticancer activity against a number of human solid tumor cell-line xenograft mouse models, including phosphatase and tensin homolog mutant endometrial, breast, and renal cell carcinomas. Findings from these nonclinical pharmacology studies suggest that TAK-228 has therapeutic potential as a p.o. administered mTORC1/2 inhibitor for the treatment of cancers associated with dysregulated activation of the PI3K/AKT/mTOR pathway.

2.2 Safety Pharmacology

TAK-228 has a low potential to affect the human ether-à-go-go related gene potassium ion channel and does not affect cardiovascular parameters in telemeterized monkeys.

2.3 Drug Metabolism and Pharmacokinetics

TAK-228 displayed dose-proportional plasma exposures, a moderate propensity to cross the blood-brain barrier, and modest (70.5%) human plasma protein binding. TAK-228 distributed mainly to the plasma fraction of human blood. There was no obvious concentration-dependent red blood cell distribution of TAK-228 in human blood.

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

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 glucuronidation (approximately 22%) and an unidentified non-uridine diphosphate glucuronosyl 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 35%), 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.

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.

The findings in rat and monkey repeat-dose toxicology studies with TAK-228, including bone marrow and lymphoid depletion; gastrointestinal (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. TAK-228 does not present a genotoxic risk and was negative for phototoxicity in the 3T3 fibroblast assay.

3. SUMMARY OF EFFECTS IN HUMANS

TAK-228 is in clinical development as a single agent in 3 phase 1 studies including Study INK128-001 in patients with advanced solid malignancies; Study INK128-002 in patients with multiple myeloma (MM), non-Hodgkin lymphoma, and Waldenström's macroglobulinemia (WM); and Study C31002 to measure the effect of TAK-228 on heart rate-corrected QT interval (QTc) in patients with advanced solid malignancies. It has also been investigated in combination with paclitaxel (with or without trastuzumab) in patients with advanced solid tumors (phase 1 Study INK128-003). TAK-228 is also being tested in combination with exemestane or fulvestrant in women with ER-positive, HER2-negative advanced or mBC (phase 1b/2 Study C31001) and in combination with the oral PI3Kα inhibitor TAK-117 (previously MLN1117; Study C32001).

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

As of 2015, a new TAK-228 capsule containing milled active pharmaceutical ingredient (API) was developed to allow scaled-up production. The milled API could result in a faster absorption profile with possibly higher maximum concentration (Cmax), which could present a different safety profile compared to the previous unmilled API capsules. Therefore, an additional study, a Phase I open label study to evaluate the safety, tolerability, and PK of TAK-228 as a single agent and in combination with paclitaxel in adult patients with advanced non-hematological malignancies (study TAK-228-1004), was designed to determine the recommended phase 2 dose (RP2D) for single-agent milled TAK-228 (QD and QW) and QD×3days per week in combination with paclitaxel, as well as the effect of high-fat meal on the PK of milled API.

Table 2 below summarizes TAK-228 doses, schedules, and API studied as well as evaluable PK population studies in all these studies. Details on PK and safety information for each study are available in the current IB edition.

TABLE 2 Summary of TAK-228 Clinical Studies

Study No.; Phase	Study Design	Dose (Schedule)	Evaluable PK Population
INK128-001 Phase 1	Multiple ascending doses in patients with advanced solid malignancies. (unmilled)	*TAK-228* 2, 4, 5, 6, and 7 mg (QD) 7, 10, 15, 20, 30, and 40 mg (QW) 6, 9, 12, 16, and 20 mg (QD×3d QW) 7, 10, and 13 mg (QD×5d QW)	106
INK128-002; Phase 1	Multiple ascending doses in patients with relapsed or refractory multiple myeloma or WM. (unmilled)	*TAK-228* 2, 4, 6, and 7 mg (QD) 9 and 12 mg (QD×3d QW)	39
INK128-003; Phase 1	Multiple ascending doses +paclitaxel (80 mg/m^2) in patients with advanced solid malignancies (a) (unmilled)	*TAK-228* 6, 7, 8, 9, and 10 mg (QD×3d QW) 7 mg (QD×5d QW) 30, 40 mg (QW)	47
MLN0128-1004; Phase 1	Open-label, ±paclitaxel; food effect on TAK-228 PK (milled vs unmilled); food effect on PK of TAK-228 (milled) ±paclitaxel	*TAK-228 (milled/unmilled)* 4 mg (QD) 20, 30 mg (QW) *TAK-228+paclitaxel*: 6 mg (3 QD×3d)+paclitaxel (80 mg/m^2 on Days 1, 8, and 15)	39
C31001; Phase 1b/2	TAK-228 (milled/unmilled) +exemestane or fulvestrant	*TAK-228+exemestane or fulvestrant (patients continue prestudy regimen)* 5 mg (QD, unmilled) 3 or 4 mg (QD, milled)	18
C31002; Phase 1	TAK-228 effect on QTc interval in patients with advanced solid tumors (unmilled)	*TAK-228* 40 mg	43

Data are preliminary for ongoing studies. Data cutoff date: 09 Dec 2015.

Abbreviations: ECG=electrocardiogram, PK=pharmacokinetic(s), QD=once daily, QD×3d QW=once daily for 3 consecutive days followed by a 4-day dosing holiday every week, QD×5d QW=once daily for 5 consecutive days followed by a 2-day dosing holiday every week, QT=interval on ECG between the start of the Q wave and end of the T wave, QTc=QT interval corrected for heart rate, QW=once weekly, WM=Waldenström macroglobulinemia.

(a)TAK-228 doses were administered in 4-week (28-day) cycles in combination with 80 mg/m^2 paclitaxel (dosed once weekly for 3 weeks [Q3W]).

3.1 Pharmacokinetics

PK data from Studies INK128-001, INK128-002, and INK128-003 indicate that TAK-228 exhibits fast oral absorption (time to Cmax [tmax] generally between 1-4 hours after dosing) and dose-linear PK with a mean plasma half-life of approximately 8 hours. TAK-228 does not accumulate meaningfully in plasma when dosed as frequently as once daily under any of 4 tested dosing regimens. The PK data of TAK-228 were generally consistent across 3 phase 1 studies, suggesting no appreciable difference in the PK of TAK-228 among patients with advanced solid tumors or patients with MM or WM.

There were no meaningful differences in the PK of TAK-228 when administered 24 hours after a 30-minute intravenous (IV) infusion of 80 mg/m^2 paclitaxel (Study INK128-003) compared with single-agent TAK-228 (Studies INK128-001 and INK128-002). The PK of paclitaxel also remained generally unaffected by TAK-228 co-administration, indicating the lack of a PK interaction between TAK-228 and paclitaxel.

There were no readily apparent differences in either the Cmax or area under the curve (AUC) of 4 mg TAK-228 unmilled or milled capsules when administered under fasted conditions.

Compared to the fasted state, when 4 mg of milled TAK-228 API was administered following a standard high-fat breakfast, there was an approximately 38% reduction in Cmax and a delay in tmax (median tmax 6 hours [fed] vs. 2 hours [fasted]), but there was no meaningful change in AUC. The differences observed when TAK-228 was dosed under fed versus fasted conditions may help explain the different MTDs determined for TAK-228 QD dosing in Study INK128-001 compared with study MLN0128-1004.

There were no readily apparent differences in the PK of TAK-228 when administered in conjunction with either 25 mg exemestane or with 500 mg fulvestrant.

3.2 Safety

Among the 438 patients who had received ≥1dose of study drug as of the clinical data cutoff date (09 December 2015), 20 deaths had occurred within 30 days of the last dose. Of these fatal events, 1 death (ventricular fibrillation and cardiac arrest in Study INK128-001) was considered related to TAK-228 treatment.

Across the studies and regardless of causality or dosing regimen, the most common TEAEs included nausea, fatigue, hyperglycemia, vomiting, diarrhea, stomatitis, and decreased appetite.

4. CLINICAL SUMMARY OF SAFETY

4.1 Special Warnings and Precautions for Use

4.1.1 Insulin and Glucose Levels

Hyperglycemia and hyperinsulinemia are known toxicities associated with inhibition of mTOR and related pathways based on nonclinical studies. A rise in fasting plasma glucose has been observed as early as 1 to 2 days following oral administration of TAK-228. However, most episodes of hyperglycemia occurred within the first 60 days after initiation of treatment with TAK-228, have been either grade 1 or grade 2, and have responded quickly to oral metformin. Daily in-home glucose monitoring and early initiation of hyperglycemia treatment are essential. For patient self-monitoring of blood glucose, a finding of fasting blood glucose (FBG) ≥ 150 mg/dL measured by glucometer would initiate closer monitoring of serum glucose and possible intervention. Patients with grade 1 hyperglycemia (fasting serum glucose [FSG] > the upper limit of normal [ULN] ≤ 160 mg/dL) are treated with oral hypoglycemic agents (e.g., metformin), and patients with ≥ grade 2 hyperglycemia (FSG > 160 mg/dL) are treated aggressively with oral hypoglycemic agents and/or insulin as clinically indicated. Daily home monitoring and early treatment have resulted in good control of glucose levels for the majority of TAK-228-treated patients who developed hyperglycemia.

4.1.2 Cardiac Effects

Cardiac events (including QTc prolongation and arrhythmias) have been infrequently observed in clinical studies of TAK-228. As of December 2015, there has been 1 report of ventricular fibrillation and cardiac arrest post-dose that had a fatal outcome and was assessed as related to TAK-228. Recent results of the C31002 phase 1 study indicate that treatment with TAK-228 is not associated with clinically meaningful effects on the overall electrocardiographic safety profile. Routine cardiac monitoring with baseline and EOT electrocardiograms (ECGs) and physical examination constitute the core cardiac safety monitoring in all TAK-228 studies.

Preliminary results from a dedicated study of the effects of TAK-228 on the QTc interval (study C31002) show lack of clinically relevant effects on QTc interval, PR and QRS intervals, minimal effects on heart rate, and absence of treatment-emergent ECG morphology findings and therefore treatment with TAK-228

is not associated with clinically meaningful effects on the overall electrocardiographic safety profile (further details available in the current IB version).
For subjects showing any signs of cardiac instability after TAK-228 dosing, additional monitoring onsite before clinic discharge should be considered.

4.1.3 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 AE 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. Patients should 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. Each dose of TAK-228 should be taken orally with 8 ounces (240 ml) of water.

Baseline macroscopic urinalysis and routine serum chemistries along with other safety laboratory assessments are performed in all TAK-228 studies. Additionally, microscopic urinalysis, 12-hour urine collection, and evaluation of spot urine electrolytes, protein and creatinine, and serum chemistry should be performed when the serum creatinine is ≥ grade 1, according to NCI CTCAE version 4.03, to further evaluate possible etiologies for the renal dysfunction.

4.1.4 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 patients have required pulse systemic steroids, dose reduction, and/or study treatment discontinuation.

4.1.5 Pneumonitis

Pneumonitis is a known potential risk of mTOR inhibitors. Early recognition, prompt intervention, and a conservative risk management approach are recommended for pneumonitis management in patients receiving rapalog therapy and TAK-228. Symptoms of pneumonitis will be closely monitored in all study patients.

4.2 Interactions With Other Medicaments and Other Forms of Interaction

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

Potential risks of combination TAK-228 and tamoxifen include fatigue, stomatitis, rash, anorexia, and diarrhea. This data is based on the combination of tamoxifen with everolimus, an mTORC1 inhibitor.[2]

5. TAMOXIFEN[38]

Drug Name: Tamoxifen
Synonym(S): Tam, Tamoxifene
Common Trade Name(S): APO-TAMOX®, GEN-TAMOXIFEN®, NOLVADEX-D®, NOVO-TAMOXIFEN®, TAMOFEN®

Classification: endocrine antihormone

Mechanism of Action: Tamoxifen and several of its metabolites are thought to act as estrogen antagonists. Tamoxifen competitively binds to ERs on tumor cells and other tissue targets, producing a nuclear complex that decreases DNA synthesis. This mechanism appears to have cytostatic effects, causing G0 and G1 cell cycle arrest. Tamoxifen may also have cytotoxic activity; tamoxifen may induce apoptosis independent of ER expression. Tamoxifen acts as an estrogen agonist on endometrium, bone, and lipids.

TABLE 3 TAMOXIFEN PHARMACOKINETICS

Interpatient Variability	Considerable variation in serum concentrations after single doses and at steady state; genetic polymorphism may influence the efficacy and toxicity of tamoxifen and its metabolites	
Oral Absorption	Well absorbed	
	time to peak plasma concentration	3-7 hours
Distribution	high concentrations found in uterus and breast tissue	
	Cross blood brain barrier?	yes
	Volume of distribution	20 L/kg
	Plasma protein binding	99%
Metabolism	Metabolized by hepatic cytochrome1 P450; major CYP3A4, 2C8/9, 2D6; minor 2A6, 2B6, 2E1	
	Active metabolite(s)	N-desmethyltamoxifen, 4-hydroxytamoxifen, and 4-hydroxy-N-desmethyltamoxifen (endoxifen)
	Inactive metabolite(s)	Yes
Excretion	Extensive enterohepatic circulation	
	Urine	9-13%
	Feces	26-65%, excreted into bile
	Terminal half-life	5-7 days, range 3-21 days; major metabolite 9-14 days
	Clearance	No information found

Uses:

Primary uses: Brain tumors, Breast cancer, Melanoma, Soft tissue sarcoma

Other uses: Carcinoid tumor, Endometrial cancer, Pancreatic cancer

Special Precautions:

Carcinogenicity: Tamoxifen is carcinogenic.

Mutagenicity: Tamoxifen is not mutagenic in the Ames test or mammalian *in vivo* mutation test. The clastogenicity of tamoxifen is not known.

Fertility: Tamoxifen may cause menstrual cycle disturbances, including infrequent or light menstruation and amenorrhea. Tamoxifen does not induce menopause. Premenopausal women should be advised not to become pregnant while taking tamoxifen. Tamoxifen has been used to treat infertility.

Pregnancy: Tamoxifen has been assigned to FDA Pregnancy Category D. There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk (e.g., if the drug is needed for a life-threatening situation or a serious disease for which safer drugs cannot be used or are ineffective).

Breastfeeding: Breastfeeding is not recommended while using tamoxifen because of its potential secretion into breast milk. Tamoxifen may inhibit lactation.

Special populations: The risk of tamoxifen-associated serious AEs (SAEs) is higher in patients older than 50 years of age. Women of childbearing potential should initiate tamoxifen during menstruation; barrier or non-hormonal contraceptives should be used and pregnancy avoided for 2 months after tamoxifen is discontinued. Porphyric patients must avoid tamoxifen, as tamoxifen has been associated with acute attacks of porphyria.

Side Effects:

Table 4 below includes AEs that presented during drug treatment but may not necessarily have a causal relationship with the drug. Because clinical trials are conducted under very specific conditions, the AE rates observed may not reflect the rates observed in clinical practice. AEs are generally included if they were reported in more than 1% of patients in the product monograph or pivotal trials and/or determined to be

clinically important. When placebo-controlled trials are available, AEs are included if the incidence is > 5% higher in the treatment group.

TABLE 4 TAMOXIFEN SIDE EFFECTS

Organ site	Side effect
Allergy/immunology	hypersensitivity reactions (<3%), vasculitis
Bone marrow/febrile neutropenia	Myelosuppression; anemia, leukopenia, neutropenia, thrombocytopenia, transient (<10%)
Cardiovascular	QT prolongation, cardiovascular event (4%, severe 1%), hypertension (7-11%), ischemic heart disease (1-3%, severe 0.6%), thromboembolic events (2-5%, severe 1-2%)
Constitutional symptoms	Fatigue (4-24%, severe 2%), sweating (6-18%, severe 3%), weight gain (8-9%), weight loss (23%)
Dermatology/skin	Alopecia (<5%), cutaneous lupus erythematosus, nail changes (3%), porphyria cutanea tarda, radiation recall, rash (<13%), skin changes (6-19%), Stevens-Johnson syndrome, erythema multiforme, bullous pemphigoid (<1%)
Endocrine	Hot flashes (25-81%, severe 4%)
Gastrointestinal	Anorexia (1-3%) constipation (1-8%), diarrhea (2-7%, severe 0.04%), dyspepsia (6%) dry mouth (2%) nausea (5-26%, severe 0.7%), vomiting (2%)
Hemorrhage	Hemorrhage, vaginal bleeding (2-23%, severe 0.1-0.3%)
Hepatobiliary/ pancreas	Cholestasis (<1%), gallstones; generally occurs after 2-3 years of treatment, pancreatitis (<1%), liver dysfunction, hepatitis (<1%)
Infection	Urinary tract infection (10%), vulvovaginal candidiasis (4%)
Lymphatics	Peripheral edema (8-11%)
Metabolic/laboratory	Elevated creatinine, hypercalcemia (< 1%), altered lipid profile; decreased total and LDL cholesterol, decreased HDL cholesterol, hypercholestremia (3%), increased triglycerides (≤ 1%)
Musculoskeletal	Arthritis (14%), arthrosis (4%) favorable effect on bone mass, fractures (4-8%), osteoporosis (6-7%)
Neurology	Anxiety (6%), depression (4-12%, severe 0.2%), dizziness (8-12%, severe 0.6%), ischemic cerebrovascular events (1-3%, severe 1%), insomnia (6-17%, severe 1%), paresthesia (5%)
Ocular/visual	Cataracts (<7%), corneal changes (≤ 0.1%), retinopathy (≤1%), vision changes (6%, severe 0.4%)
Pain	abdominal pain (7-9%), arthralgia/myalgia (4-29%, severe 0.4-0.9%), back pain (10%), bone pain (6%), breast pain (6%), chest pain (5%), cramps (4%, severe 0.2%), headache (2-16, severe 0.8%), pain not specified (16%), tumor pain
Pulmonary	Cough (4-10%), pharyngitis (<1%), pneumonitis (<1%)
Renal/genitourinary	Endometrial polyps, hyperplasia, endometriosis (≤1%), ovarian cysts (<3%), pruritus vulvae (<1%), vulvovaginitis (5%), urinary incontinence (4%), uterine fibroids (≤1%), non-infectious vaginal discharge, leukorrhea (9-13%), vaginal dryness (<3%)
Secondary malignancy	Endometrial cancer (0.8%), uterine sarcoma
Sexual/reproductive function	Impotence (<1%), menstrual dysfunction, priapism
Syndromes	Flu syndrome (6%), tumor flare (<10%)

Hot flashes are one of the most common AEs reported in women taking tamoxifen, but are rarely severe. If severe, they may be controlled in some patients by a decreased or divided dose. Patients who have their sleep interrupted by drenching night sweats may benefit by taking their tamoxifen in the morning. Several medications have been shown to decrease the frequency and severity of hot flashes. Occasionally tamoxifen must be discontinued due to severe hot flashes that significantly decrease quality of life.

Tamoxifen flare response: A transient increase in bone pain, local disease flare (increase in size of preexisting lesions, swelling, and redness), and/or hypercalcemia may occur at the initiation of therapy in patients with metastatic disease. Serum calcium should be evaluated in any patient with extensive bony metastases who has symptoms suggestive of hypercalcemia. The so-called tamoxifen flare response may be a favorable sign, although hypercalcemia may require treatment.

Endometrial changes: Tamoxifen has a stimulant effect on the endometrium, possibly by acting as a partial estrogen agonist. Tamoxifen use has been associated with an increased incidence of endometrial changes, including hyperplasia, polyps, uterine fibroids, and endometriosis.

Uterine malignancies associated with tamoxifen are typically adenocarcinomas of the endometrium; uterine sarcomas, an endometrial cancer with poor prognosis, have also been rarely reported. The relative risk of endometrial cancer increases with the duration of tamoxifen therapy; this relative risk is small and must be weighed against the potential benefits of tamoxifen. Patients receiving or who have received tamoxifen should have routine gynecological care and should be advised to immediately report any abnormal gynecologic symptoms, such as menstrual irregularities, abnormal vaginal bleeding or discharge, and pelvic pain and pressure. Imaging, including endovaginal US and/or endometrial biopsy, may be necessary to rule out malignancy.

Ocular changes (retinopathy, corneal opacities, and decreased visual acuity) have been reported in patients receiving tamoxifen. A modest increase in the risk of developing cataracts has been associated with tamoxifen treatment. The relationship between tamoxifen dose and cataract formation is not known. Cataract formation may be due to tamoxifen-mediated inhibition of chloride channels in the lens. Macular degeneration does not appear to predispose subjects to tamoxifen-related ocular toxicity nor does tamoxifen accelerate progression of macular degeneration. Patients receiving or who have received tamoxifen should be questioned about symptoms of ocular toxicity during follow-up and should seek prompt medical attention for changes in vision.

Thromboembolic events, including deep vein thrombosis, stroke, and pulmonary embolism, are increased with tamoxifen use. Use tamoxifen with caution in individuals with a history of thromboembolic events, particularly those not receiving systemic anticoagulation therapy.

Hepatotoxicity usually consists of transient asymptomatic elevation of hepatic enzymes. However, more serious liver abnormalities, including fatty liver, cholestasis, and hepatitis, have occurred infrequently; rarely fatalities have been reported.

Lipid profile: Tamoxifen favorably affects lipid profiles by decreasing total and low-density lipoprotein cholesterol concentrations; this effect does not translate to a reduced risk of ischemic heart disease. Less favorably, tamoxifen appears to moderately decrease high-density lipoprotein cholesterol concentrations and increase triglyceride levels. Rarely, cases of pancreatitis have occurred. Periodic monitoring of plasma cholesterol and triglyceride concentrations is advised for patients taking tamoxifen who have preexisting hyperlipidemias or other clinical indications.

Myelosuppression has been reported with tamoxifen. Temporary decreases in platelet and leukocyte counts may occur. Hemorrhagic tendencies are uncommon, and platelet counts have returned to normal without treatment interruption. If myelosuppression is suspected, monitor complete blood counts. Use tamoxifen with caution in patients with thrombocytopenia or leukopenia.

Bone mass: Tamoxifen generally has a favorable effect on bone mass. Tamoxifen reduces bone resorption and decreases bone turnover as manifested by reductions in bone turnover markers and increases in bone density. Tamoxifen acts mainly on trabecular bone, such as the lumbar spine, and has little effect on cortical bone. The effect of tamoxifen on bone density may depend on menopausal status, as premenopausal women have demonstrated a loss of bone mineral density of the lumbar spine and hip. Further information is needed to evaluate the long-term effects of tamoxifen on the risk of osteoporosis and fracture.

TABLE 5 TAMOXIFEN DRUG INTERACTIONS

Agent	Effect	Mechanism	Management
Aldeskleukin	Increased risk of hypersensitivity	Unknown	Monitor for signs and symptoms of hypersensitivity reactions
Aminoglutethimide	Decreased tamoxifen and its active metabolites concentration	Increased metabolism	Avoid concurrent use
Anastrozole	Tamoxifen decreases plasma anastrozole level by 27%, but has no significant effect on estrogen suppression by anastrozole; anastrozole has no significant effects on the pharmacokinetics of tamoxifen.		
Bexarotene	35% decrease in tamoxifen plasma concentrations	Unknown; likely be due to induction of CYP3A4 by bexarotene	Clinical significance unclear; consider alternate agent (s)
Bromocriptine	Increased tamoxifen concentrations	Decreased metabolism of tamoxifen	Caution
Cyclophosphamide	Decreased cytotoxic effects of cyclophosphamide	Unknown	Avoid concurrent use; start adjuvant tamoxifen after chemotherapy is complete
Cytotoxic agents	Increased risk of thromboembolic events	Unknown	Caution
Doxorubicin	Decreased cytotoxic effects of doxorubicin	Unknown	Avoid concurrent use; start adjuvant tamoxifen after chemotherapy is complete
Estrogens	May interfere with therapeutic effect of tamoxifen	May counter the estrogen suppression effect of tamoxifen	*See below

Exemestane	No significant effects on tamoxifen or exemestane pharmacokinetics		
Fluorouracil	Decreased cytotoxic effects of fluorouracil	Unknown	Avoid concurrent use; start adjuvant tamoxifen after chemotherapy is complete
Grapefruit juice	May affect bioavailability of tamoxifen and its active metabolite	May inhibit CYP3A4 metabolism of tamoxifen in intestinal wall	†See below
Letrozole	Tamoxifen decreases plasma letrozole level by 38%, but has no significant effect on estrogen suppression by letrozole; letrozole has no effects on the pharmacokinetics of tamoxifen and its major metabolites		
Mitomycin	Increased risk of hemolytic uremic syndrome	Unknown	Avoid concurrent use
Paroxetine and other selective serotonin inhibitors that inhibit CYP2D6	Reduced tamoxifen active metabolite concentrations	Inhibits CYP2D6 metabolism of tamoxifen	‡See below
Rifamycins (e.g. rifabutin, rifampin, rifapentine)	Reduced tamoxifen concentrations; potentially increased levels of N-desmethyltamoxifen metabolite, and subsequently endoxifen (active metabolite)	Induced CYP3A4 metabolism of tamoxifen	No alteration of efficacy expected; clinical impart is unknown
Thyroid function tests	Elevated thyroid hormone levels (T4 and T3)	Increased thyroxine-binding globulin	None, thyroid function does not appear to be affected
Warfarin	Delayed, major, possible; increased anticoagulant effect	Unknown	Monitor prothrombin time, adjust warfarin dose accordingly

***Estrogen use with tamoxifen**: Hormone replacement therapy is not recommended following ER-positive breast cancer or while on tamoxifen. Postmenopausal symptoms can cause considerable distress to patients; replacement therapy may be considered if other treatment options fail. If estrogen is used, prescribe the lowest dose to relieve symptoms, monitor patient carefully, and consider short term use. For vaginal complaints such as dyspareunia, dryness, and sexual dysfunction, REPLENS®, a long-lasting vaginal moisturizer, can be tried. If ineffective, low dose topical estrogen may then be considered. ESTRING® produces a local effect with systemic levels measurable only for the first 24 hours of the three-month ring. PREMARIN® cream can be used but may have variable systemic levels related to the absorption through the vaginal tissues. The potential risks and benefits should be discussed, the lowest dose to relieve symptoms should be used, and treatment should be assessed regularly.

†**Grapefruit juice and tamoxifen**: Grapefruit juice inhibits CYP3A4-mediated metabolism of tamoxifen in the intestine and may increase tamoxifen plasma levels. The clinical significance of a low rate of intestinal metabolism to active metabolites is unknown. Patients should be monitored for signs of tamoxifen toxicity.

‡**Antidepressant use with tamoxifen**: The metabolism of tamoxifen to active 4-hydroxy-N-desmethyl-tamoxifen (endoxifen) is inhibited by the selective serotonin reuptake inhibitor (SSRI) paroxetine, a potent CYP2D6 inhibitor. Other SSRIs that inhibit CYP2D6 also inhibit tamoxifen metabolism. The magnitude of reduction in endoxifen plasma concentration associated with CYP2D6 inhibitors also depends on variations in CYP2D6 genotypes. The minimally active levels of tamoxifen and its metabolites are not known. The clinical significance of a low rate of endoxifen hydroxylation is not known; the potential benefit of antidepressant use must be weighed against the potential risk. Antidepressants that weakly inhibit or do not inhibit CYP2D6 may be considered.

List of Antidepressant and Tamoxifen Interactions:

Tamoxifen is converted to 4-hydroxytamoxifen, endoxifen, and other active metabolites by the liver enzyme CYP2D6. The efficacy of tamoxifen may vary among individuals due to genetic variation in CYP2D6 activity and coadministration of drugs that may modulate CYP2D6 activity.

SSRIs are a commonly used class of antidepressants, which inhibit CYP2D6 to varying degrees. Concurrent administration of some SSRIs and tamoxifen has been shown to lower levels of endoxifen, but not 4-hydroxytamoxifen. The clinical implications of this decline in endoxifen levels are unclear, because tamoxifen concentrations do not appear to change substantially. However, retrospective evidence presented at the 2009 American Society of Clinical Oncology meeting indicate that concomitant use of

tamoxifen and moderate/potent CYP2D6 inhibitors significantly increase the risk of breast cancer recurrence. In the SSRI subanalysis, tamoxifen in combination with moderate or potent CYP2D6 inhibitors was associated with a 25–92% greater relative risk of breast cancer recurrence (depending on duration of coexposure) compared with tamoxifen alone. Weak CYP2D6 inhibitors were not associated with increased risk of breast cancer recurrence. A recent retrospective study from Ontario also suggests that the greater risk of breast cancer recurrence with paroxetine may be associated with increased cancer death.

Drugs that could potentially reduce efficacy and thus, should be used with caution include any strong CYP2D6 inhibitor such as fluoxetine, paroxetine, chlorpromazine, miconazole, quinine, and bupropion and any moderate CYP2D6 inhibitor such as ketoconazole, trazodone, and amiodarone. The safest course of action is to avoid coadministration of tamoxifen and any of these medications. However, each patient's particular needs and circumstances should be evaluated to determine what is best for them.

Serotonin norepinephrine reuptake inhibitors such as venlaflaxine and desvenlafaxine are weak CYP2D6 inhibitors and do not lower the concentration of endoxifen. These are better choices for women taking tamoxifen who also require medication for depression or relief of hot flashes.

Appendix B lists examples of commonly used antidepressants and their association with CYP2D6 and tamoxifen.

Supply and Storage:

Tablets: Apotex, Genpharm, and Novopharm supply tamoxifen as 10 or 20 mg tablets.

AstraZeneca supplies tamoxifen as a 20 mg tablet. Selected non-medicinal ingredients: lactose.

Aventis Pharma supplies tamoxifen as 10 or 20 mg tablets. Selected non-medicinal ingredients: lactose.

Store tamoxifen at room temperature and protect from light.

Dosage Guidelines:

This protocol will use 20 mg p.o. QD. Guidelines for dosing also include consideration of absolute neutrophil count (ANC). Dosage may be reduced, delayed, or discontinued in patients with bone marrow depression due to cytotoxic/radiation therapy or other toxicities.

Adults:

Oral:	20 mg p.o. QD
Dosage in renal failure:	No adjustment required
Dosage in hepatic failure:	Adjustment required, no details found; dosing may be based on serum levels of tamoxifen and its active metabolites
Dosage in dialysis:	No adjustment required

6. STUDY RATIONALE

The recently completed phase II TAMRAD trial evaluated the benefit of adding an mTOR inhibitor (everolimus) to tamoxifen in the advanced disease setting. The trial included 211 postmenopausal women with hormone receptor-positive, HER2-negative advanced breast cancer previously treated with a non-steroidal AI in the adjuvant or metastatic setting. Patients were randomized to receive tamoxifen alone (20 mg p.o. QD) or tamoxifen plus everolimus (10 mg p.o. QD). The primary study endpoint was CBR at 6 months. Tamoxifen plus everolimus was superior to tamoxifen alone in terms of CBR (61% *vs.* 42%; P = 0.045) and TTP (8.6 *vs.* 4.5 months). Furthermore, the addition of everolimus increased overall survival by 55% (HR, 0.45; 95% CI, 0.28–0.81; exploratory log-rank P = 0.007).[2]

In the phase III randomized BOLERO 2 trial, 724 postmenopausal women with hormone receptor-positive mBC who had progressed on a non-steroidal AI (anastrozole or letrozole) were enrolled. Eligible women were randomized (2:1) to receive exemestane (25 mg QD) plus everolimus (10 mg QD) or exemestane plus placebo. Baseline characteristics were well balanced between the two study groups. The median age was 62 years, and 56% and 84% of patients had visceral involvement and hormone-sensitive disease, respectively. Previous therapy included letrozole or anastrozole (100%), tamoxifen (48%), fulvestrant (16%), and chemotherapy (68%). The primary endpoint was PFS, and the secondary endpoints included

survival, response rate, and safety. Also a preplanned interim analysis was performed by an independent data and safety monitoring committee after 359 PFS events were observed. A significant PFS benefit was observed for everolimus plus exemestane over exemestane alone. At the interim analysis, median PFS by local assessment was 6.9 months with everolimus plus exemestane and 2.8 months with placebo plus exemestane (HR for progression or death, 0.43; 95% CI, 0.35–0.54; $P < 0.001$). Median PFS by central assessment was 10.6 months and 4.1 months in the everolimus plus exemestane and placebo plus exemestane groups, respectively (HR, 0.36; 95% CI, 0.27–0.47; $P < 0.001$). The most common grade 3 or 4 AEs were stomatitis (8% in the everolimus plus exemestane group *vs.* 1% in the placebo plus exemestane group), anemia (6% *vs.* < 1%), dyspnea (4% *vs.* 1%), hyperglycemia (4% vs. <1%), fatigue (4% *vs.* 1%), and pneumonitis (3% *vs.* 0%). Based on these results, the FDA approved the use of everolimus in combination with exemestane in July 2012.

As the majority of breast cancers are ER positive, it is important to understand the underlying mechanisms leading to endocrine therapy resistance. HER2, PI3K, and mTOR have been postulated to be major pathways of treatment resistance.[39,40] Less than 10% of ER-positive breast cancers overexpress HER2 and therefore, it is not a major mechanism of escape from endocrine therapy. On the other hand, the PI3K/mTOR pathway is commonly dysregulated in ER-positive breast cancers and represents a key escape/resistance mechanism to endocrine therapy. The use of mTOR inhibitors has been explored as a front-line treatment for advanced breast cancers, but not for newly diagnosed ER-positive breast cancers. The TAMRAD and BOLERO 2 trials showed that blocking endocrine resistance mediated by PI3K and other prosurvival pathways with an mTOR inhibitor provided a significant PFS benefit compared with endocrine therapy alone or placebo.[39]

The development of new therapies has led to an overall decline in breast cancer mortality. Importantly, evidence indicates that treatment resistance and relapse are the primary causes of mortality in breast cancer. Furthermore, the number of patients who are resistant to standard hormonal/endocrine therapies far exceeds the number of patients with triple negative breast cancer. Despite this, little focus has been placed on identifying new therapeutic targets to overcome treatment resistance in the various breast cancer types. Our group has identified autophagy as a mechanism of endocrine resistance and survival in ER-positive breast cancer (unpublished data). Targeting prosurvival and endocrine resistance pathways such as mTOR and autophagy with FDA-approved inhibitors would increase the response to endocrine therapy in endocrine-resistant, ER-positive breast cancer. This proposal uses a novel approach to overcome endocrine resistance and improve endocrine therapy efficacy in newly diagnosed ER-positive breast cancer — combining endocrine therapy with blockade of prosurvival and resistance pathways in ER-positive breast cancer cells. The main scientific goal of this proposal is to target mTORC1/mTORC2 signaling pathways with TAK-228 to overcome tamoxifen resistance in ER-positive breast cancer.

Preclinical Data

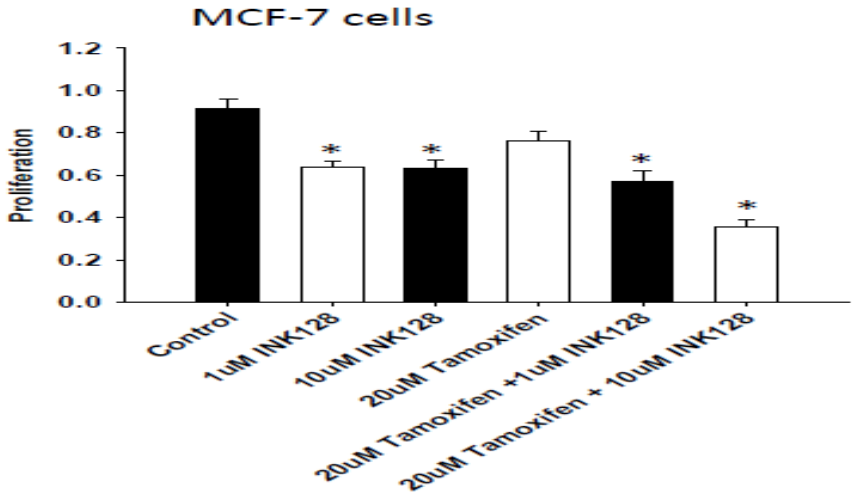


Figure 2

As shown in Figure 2, the mTOR inhibitor INK128 (TAK-228) synergistically enhances the antiproliferative effect of tamoxifen in ER-positive MCF-7 breast cancer cells. Proliferation by WST-1 assay was assessed in cells treated with INK128, tamoxifen, or both for 96 h.

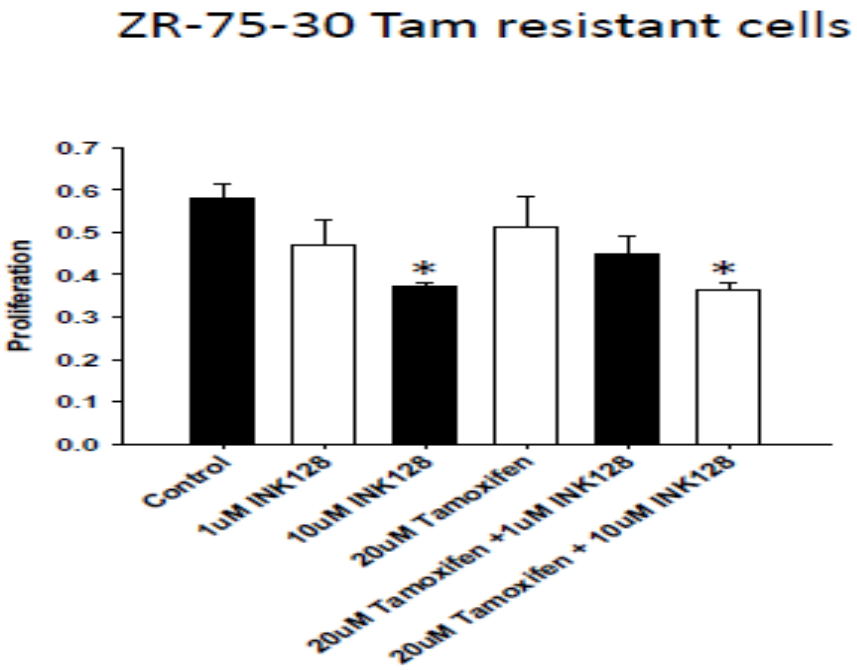


Figure 3

As shown in Figure 3, the tamoxifen-resistant ER-positive ZR75-30 cell line does not respond to tamoxifen treatment. INK128 alone at a concentration of 10 μM significantly reduced proliferation. Proliferation by WST-1 assay was assessed in cells treated with INK128, tamoxifen, or both for 96 h.

Rationale for Selected Dose and Schedules of TAK-228

RP2D for milled TAK-228 Weekly Schedule: 30 mg p.o. QW

The selected RP2D of 30 mg TAK-228 QW is based on the findings from 2 studies: Study INK128-001 and Study MLN0128-1004.

Study INK128-001 was the first-in-human study of TAK-228. This was an open label study designed to determine the MTD and to identify dose-limiting toxicities (DLTs) for p.o. 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 (31 patients), QDx3 days per week (33 patients), QDx5 days per week (22 patients), and QW (30 patients) in the dose escalation phase. Doses of 40 mg QW, 30 mg QW, and 5 mg QD were further evaluated in an additional 82 patients in the expansion phase.

Improved tolerability, including a reduced frequency of TEAEs leading to dose interruptions and modifications, respectively (30 mg QW: 24% and 41%, *vs* 40 mg QW: 19% and 77% as of data cut off 9 December, 2015) and longer duration of clinical benefit favored 30 mg QW dosing as a RP2D and schedule for further development.

Scale-up manufacturing of TAK-228 capsules 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 30 mg milled TAK-228 QW was further evaluated and confirmed in Study MLN0128-1004 in which a total of 14 patients were enrolled and assigned, sequentially, to 2 QW dosing cohorts: 20 mg QW and 30 mg QW milled TAK-228 (see Table 6 below).

TABLE 6 Dose-Limiting Toxicity Observed with Milled TAK-228 QW in Study MLN0128-1004

Dose of Milled TAK-228	Number of Evaluable Patients	Patients with DLTs observed in Cycle 1
20 mg QW	6	None
30 mg QW	6	None

DLT = dose-limiting toxicity; QW = once weekly.

As none of the patients in either dose cohort experienced DLT in Cycle 1, a dose of 30 mg milled TAK-228 QW, dosed in empty-stomach conditions was selected as RP2D for further development. No clinically meaningful differences in PK of TAK-228 were noted between the unmilled TAK-228 (Study INK128-001) and milled TAK-228 (Study MLN0128-1004) when given QW (further details available in the current IB version).

Innovation

This proposal is innovative in various aspects. Firstly, it targets the ER with an FDA-approved compound (tamoxifen). Tamoxifen competitively binds to ERs on tumor cells and other tissue targets, producing a nuclear complex that decreases DNA synthesis and inhibits estrogen effects. Secondly, our proposal is highly relevant given that mTOR inhibitors are currently being investigated for the treatment of ER-positive endocrine-resistant breast cancer. Here, we propose an open label phase II study of TAK-228 in

combination with tamoxifen in patients with newly diagnosed ER–positive breast cancer. Use of TAK-228 plus tamoxifen is supported by strong data showing that this combination decreases ER-positive breast cancer cell proliferation and increases pCR in patients with newly diagnosed ER–positive breast cancer. Thirdly, the correlation of specific molecular markers (ER; Ki67; mitotic index; apoptosis; levels of S6K1, 4EBP-1, eukaryotic translation initiation factor (EIF)4E, EIF4G, and EIF4A; levels of phosphorylated S6K1, p53, p63, and p73; and p73 and p63 gene signatures) with clinical parameters such as tumor response will help define a biomarker signature associated with p63/p73 and/or PI3K/AKT dependence and NFkB in ER-positive breast cancers and identify new therapeutic options for patients with ER-positive breast cancers. All tissues collected during the study will be stored for future analyses including genomic analyses. Tumor biopsy specimens obtained at the time of first diagnosis and week 6 of treatment will be correlated with the surgical specimen. An additional biopsy will be obtained from patients whose disease progresses while on treatment.

7. STUDY OBJECTIVES

7.1 Primary Objective

- To evaluate Ki67 before and after treatment with the mTORC1/2 inhibitor TAK-228 plus the non-steroidal agent tamoxifen in patients with newly diagnosed ER-positive, HER2-negative breast cancer.

7.2 Secondary Objectives

- To evaluate the pCR rate after treatment with TAK-228 plus tamoxifen in patients with newly diagnosed ER-positive, HER2-negative breast cancer. pCR is defined as the absence of residual invasive and in situ cancer on hematoxylin and eosin evaluation of the complete resected breast specimen and all sampled regional lymph nodes following completion of neoadjuvant systemic therapy (i.e., ypT0 ypN0 in the current American Committee on Cancer staging system).
- To evaluate the PEPI score after treatment with TAK-228 plus tamoxifen in patients with newly diagnosed ER-positive, HER2-negative breast cancer.
- To assess the toxicity and safety of TAK-228 plus tamoxifen in patients with newly diagnosed ER-positive, HER2-negative breast cancer.

7.3 Tertiary/Exploratory Objectives

- To determine the plasma PK of the TAK-228 plus tamoxifen combination.
- To assess the correlation between pCR to TAK-228 plus tamoxifen and changes in tissue-based markers including Ki67, p53/p63/p73, PI3K/AKT/mTOR, and NFkB pathways in ER-positive, HER2-negative breast tumors.
- To assess tumor mutational status to identify predictors of response to the TAK-228 plus tamoxifen combination.
- To evaluate Oncotype DX recurrence score before and after treatment with TAK-228 plus tamoxifen.

8. STUDY ENDPOINTS

8.1 Primary Endpoint

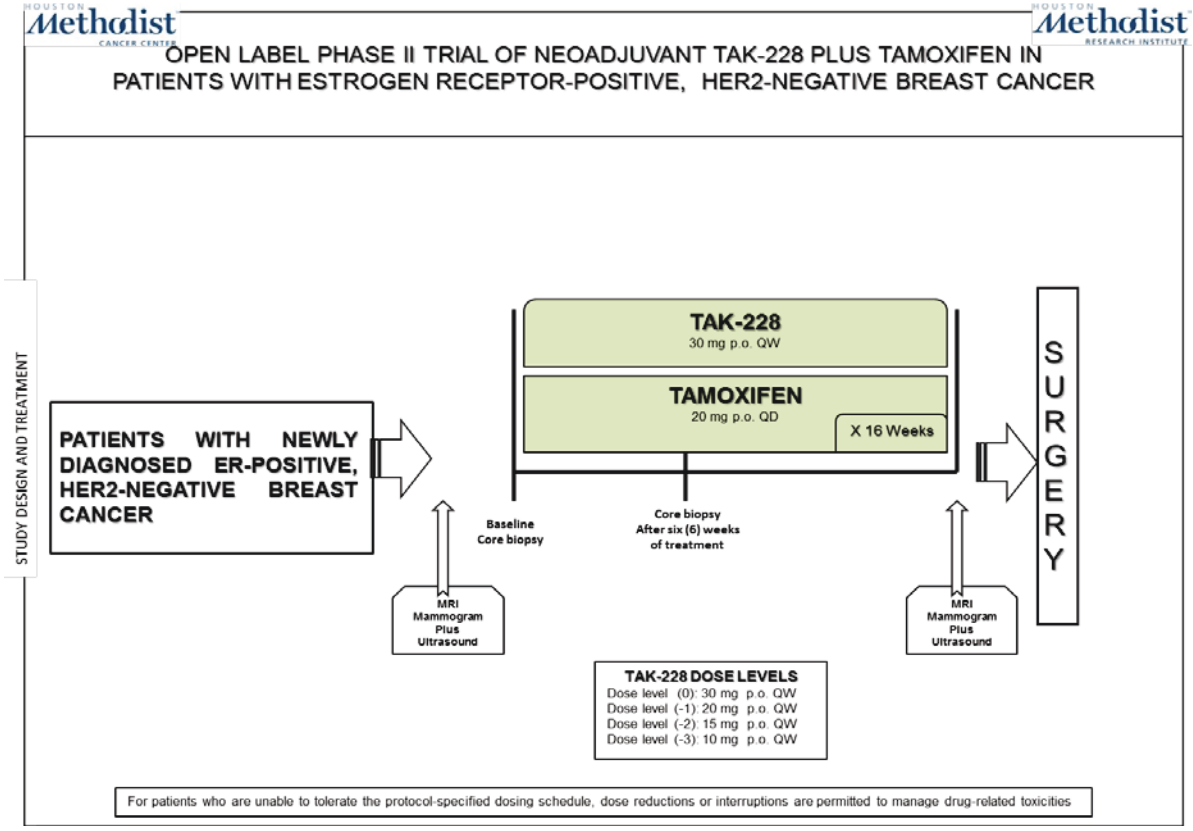
The primary endpoint of this protocol is the change in Ki67 after treatment with TAK-228 plus tamoxifen in patients with newly diagnosed ER-positive, HER2-negative breast cancer.

8.2 Secondary Endpoints

Secondary endpoints include pCR rate, PEPI score, toxicity, and safety of TAK-228 plus tamoxifen in patients with newly diagnosed ER-positive, HER2-negative breast cancer.

8.3 Tertiary/Exploratory Endpoints

The exploratory endpoints include assessment of plasma PK of the TAK-228 plus tamoxifen combination and correlative markers in biopsies. The correlation between pCR to TAK-228 plus tamoxifen and changes in Ki67, p53/p63/p73, PI3K/AKT/mTOR, and NFkB pathways in ER-positive, HER2-negative breast tumors will also be assessed. Tumor mutational status will also be assessed to identify predictors of response to the TAK-228 plus tamoxifen combination. Oncotype DX recurrence score will be evaluated before and after treatment with TAK-228 plus tamoxifen.



9. STUDY DESIGN

9.1 Overview of Study Design

Open label phase II clinical trial to determine the efficacy (change in Ki67, pCR rate, and PEPI score), toxicity, and safety of TAK-228 plus tamoxifen in patients with newly diagnosed ER-positive, HER2-negative breast cancer.

Figure 4

9.2 Number of Patients

The calculated sample size is 35 patients, with enrollment considered the day the informed consent form (ICF) is signed.

9.3 Duration of Study

Patients will receive TAK-228 QW and tamoxifen QD for 16 weeks. Screening for inclusion in the study will be done within 28 days prior to the day 1 dose of TAK-228 and tamoxifen. Baseline vital signs, labs, physical exam, and biopsy and tumor tissue collection will be done within 7 days prior to the day 1 dose of TAK-228 and tamoxifen. Visits will occur at weeks 1, 3, 4, 6, 8, 12, and 16 and at end of treatment (7 to 10 days after the final dose of study treatment).

10. STUDY POPULATION

The study is designed for patients with newly diagnosed ER-positive, HER2-negative breast cancer. Written informed consent is required before performing any study-specific tests or procedures. Signing of the Informed Consent Form can occur outside the 28-day screening period. All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before study entry. Results of standard-of-care tests or examinations performed prior to obtaining informed consent and within 28 days prior to study entry (except where otherwise specified) may be used for screening assessments rather than repeating such tests. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

10.1 Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be enrolled in the study:

1. Female or male ≥ 18 years of age.
2. Newly diagnosed ER-positive, HER2-negative breast cancer. ER-positive is defined as ≥ 1% immunohistochemical (IHC) staining of any intensity. HER2 test result is negative if a single test (or both tests) performed show:
   - IHC 1+ or 0
   - In situ hybridization negative based on:
     - Single-probe average HER2 copy number < 4.0 signals/cell
     - Dual-probe HER2/CEP17 ratio < 2 with an average HER2 copy number < 4.0 signals/cell.
3. Patients with stage II-III breast cancer are eligible if they are deemed appropriate for neoadjuvant endocrine therapy by the referring or treating medical oncologist. Patients with stage I disease are eligible if they are deemed borderline candidates for breast conservation and the treating surgeon recommends preoperative therapy to increase the chances of breast conservation.
4. Eastern Cooperative Oncology Group performance status of ≤ 1 (see Appendix C).
5. Female patients who:
   - Are postmenopausal for at least 1 year before the screening visit, OR
   - Are 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 the ICF through 90 days (or longer, as mandated by local labeling [e.g., United Surgical Partners International, summary of product characteristics, etc.] after the last dose of the study drugs, 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 condom should not be used together).

6. Male patients, even if surgically sterilized (i.e., status post-vasectomy), who:
   - Agree to practice highly effective barrier contraception during the entire study treatment period and through 120 days after the last dose of the study drugs, 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 condom should not be used together).
   - Agree not to donate sperm during the course of this study or within 120 days after receiving their last dose of the study drugs;
7. Screening clinical laboratory values as specified below:
   a) Bone marrow reserve consistent with: ANC ≥ 1.5 x 10^9/L, platelet count ≥ 100 x 10^9/L, and hemoglobin ≥ 9 g/dL (without transfusion) within 1 week preceding the administration of the study drugs;
   b) Hepatic status: Serum total bilirubin ≤ 1 x ULN (in the case of known Gilbert's syndrome, a higher serum total bilirubin [> 1.5 x ULN] is allowed), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 1.5 x ULN, and alkaline phosphatase ≤ 1.5 x ULN;
   c) Renal status: Creatinine clearance ≥50 mL/min based on Cockcroft-Gault estimate or based on urine collection (12 or 24 hour);
   d) Metabolic status: HbA1c < 7.0%, FSG ≤ 130 mg/dL, and fasting triglycerides ≤ 300 mg/dL.
8. Ability to swallow oral medications.
9. 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. Negative serum pregnancy test within 7 days prior to the administration of the study drugs for women of childbearing potential.
11. Patient must be accessible for treatment and follow-up.
12. Patient must be willing to undergo breast biopsies as required by the study protocol.

10.2 Exclusion Criteria

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

1. Any patient with metastatic disease.
2. Other clinically significant comorbidities, 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.
3. Known human immunodeficiency virus infection.
4. Known hepatitis B surface antigen-positive or known or suspected active hepatitis C infection.
5. Any serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with the completion of the protocol-specified treatment.
6. Diagnosed or treated for another malignancy within 2 years before administration of the first dose of the study drugs or previously diagnosed with another malignancy and have any evidence of residual disease. Patients with non-melanoma skin cancer or carcinoma in situ of any type are not excluded if they have undergone complete resection.
7. Breastfeeding or pregnant.

8. Manifestations of malabsorption due to prior GI surgery, GI disease, or an unknown reason that may alter the absorption of TAK-228. Patients with enteric stomata are also excluded.
9. Treatment with any investigational products within 2 weeks before administration of the first dose of the study drugs.
10. Poorly controlled diabetes mellitus (defined as HbA1c > 7%). Patients with a history of transient glucose intolerance due to corticosteroid administration may be enrolled in the study if all other inclusion criteria and none of the other exclusion criteria are met.
11. History of any of the following within the last 6 months before administration of the first dose of the study drugs:
    - 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, and ventricular tachycardia)
    - Placement of a pacemaker for control of rhythm
    - New York Heart Association Class III or IV heart failure (see Appendix D)
    - Pulmonary embolism
12. 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 antihypertensive agents to control hypertension before week 1, 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 QTc prolongation (e.g., repeated demonstration of QTc interval > 480 milliseconds or history of congenital long QT syndrome or torsades de pointes)
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 the study drugs.
14. Daily or chronic use of a proton pump inhibitor (PPI) and/or having taken a PPI within 7 days before receiving the first dose of the study drugs.
15. Patients unwilling or unable to comply with the study protocol.
16. Patients previously treated with hormonal therapy (tamoxifen, AI) or PI3K, AKT, dual PI3K/mTOR, TORC1/2, or TORC1 inhibitors.
17. Patients who are currently being treated with cancer therapy (chemotherapy, radiation therapy, immunotherapy, or biologic therapy) other than the trial therapy.
18. Patients with hypersensitivity to mTOR inhibitors or tamoxifen.

11. STUDY DRUG

11.1 Study Drug Administration

Current protocol-specific criteria for administration of TAK-228 must be met and documented before drug administration. Study drug will be administered only to eligible patients under the supervision of the investigator.

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 should drink at least 8 ounces (240 mL) of water with their TAK-228 dose. Patients should be instructed to take their study medication in the morning on each scheduled dosing day (day 1 of each week ± 1 day) and not to take more than the prescribed dose at any time. Patients should 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 on the scheduled dosing day (day 1 of each week ± 1 day), then this will be considered a missed dose. Patients should record any missed doses in their diary and resume drug administration at the next scheduled time with the prescribed dosage. Under no circumstance should 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.

Tamoxifen will be administered by the patient pursuant of a prescription obtained from the patient's pharmacy. It is to be administered with or without food QD. Patients should be instructed to take their tamoxifen at approximately the same time each day and not to take more than the prescribed dose at any time. On TAK-228 scheduled dosing days, tamoxifen should be taken in the evening (before bed). Patients should swallow the tamoxifen whole and not chew it, crush it, or manipulate it in any way before swallowing.

11.1.1 Dose Modification Guidelines

TAK-228 should be administered, unless the patient has a grade 3 or greater TAK-228-related event. Guidelines for dose interruption and dose reduction are described below.

11.1.2 Criteria for Dose Interruption During a Cycle/Visit

TAK-228 should be withheld for treatment-related toxicities that are grade 3 or higher despite supportive treatment per standard clinical practice. If the event resolves to grade 1 (or grade 2 for hyperglycemia or rash) or to baseline values within 3 weeks of interrupting treatment, then the patient may resume study treatment at a dose level reduced by 1 level.

The following non-hematologic toxicities attributed to TAK-228 would not require dose interruption:

- Grade 3 or higher nausea and/or emesis in the absence of optimal antiemetic prophylaxis (optimal antiemetic prophylaxis is defined as an antiemetic regimen that employs both a serotonin type 3 receptor antagonist and a corticosteroid given in standard doses and according to standard schedules)
- Grade 3 or higher diarrhea that occurs in the absence of optimal supportive therapy
- Grade 3 fatigue

TABLE 7 TAK-228 DOSE MODIFICATIONS

Dose Level	TAK-228 Dose	TAK-228 Number of Capsules and Strength
0	30 mg p.o. QW	Six 5 mg capsules
-1	20 mg p.o. QW	Four 5 mg capsules
-2	15 mg p.o. QW	Three 5 mg capsules
-3	10 mg p.o. QW	Two 5 mg capsules

For patients who are unable to tolerate the protocol-specified dosing schedule, dose reductions or interruptions are permitted to manage drug-related toxicities. Patients whose treatment is interrupted or permanently discontinued due to an AE including abnormal laboratory value must be followed at least once a week for 4 weeks and subsequently at 4-week intervals until resolution or stabilization of the event, whichever comes first. Dose interruptions should be reported on the appropriate Dosage Administration case report form (CRF). The maximum time allowed for toxicity-related treatment interruption is 21 days (3 weeks) from the intended dosing day. If treatment interruption is >3 weeks or more than 3 dose reductions are required, the patient must be discontinued from the study treatment. However, they will need to complete the EOT visit within 30 to 40 days after the last dose of TAK-228, and the patient will continue to be followed for toxicity.

11.2 Recommended Dose Modifications for Tamoxifen Treatment-Associated Toxicity

There are no recommendations for tamoxifen dose modifications.

11.3 Excluded Concomitant Medications and Procedures and Potential DDIs

The following medications and procedures are prohibited during the study:

- Other investigational agents including mTOR, PI3K, and AKT inhibitors
- Other anticancer therapies including chemotherapy, immunotherapy, radioimmunotherapy, targeted agents, radiation, and surgery (palliative radiation and surgery for preexisting lesions is allowed during the study)
- Systemic corticosteroids (either IV or oral steroids, excluding inhalers), unless necessary for the treatment of TAK-228-related rash.
- Antiepileptic drugs for patients with treated brain metastasis
- Concomitant administration of any PPI (e.g., omeprazole, esomeprazole, pantoprazole, lansoprazole, and rabeprazole) 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.

11.4 Permitted Concomitant Medications and Procedures

- Histamine H2 receptor antagonists (e.g., ranitidine, famotidine, and nizatidine) may be allowed, if needed, provided they are not taken within 12 hours before and within 6 hours after administration of the study drugs. Patients receiving histamine H2 receptor antagonists before enrollment must stop using these medications for at least 24 hours before their first dose of the study drugs. Cimetidine, a moderate CYP1A2 inhibitor, is not recommended as a first choice H2 receptor antagonist.

- Neutralizing antacid preparations (acid neutralizers) and calcium supplements are permitted except from 2 hours before until 2 hours after TAK-228 administration. Some anti-gas preparations may also have antacid properties and should also not be permitted from 4 hours before until 2 hours after study drug administration.
- Strong CYP1A2 inhibitors and CYP inducers should be administered with caution, at the discretion of the investigator (see Appendix E). Alternative treatments, if available, should be considered..

11.5 Precautions and Restrictions

No dietary restrictions will be imposed on study patients other than the daily fasting glucose test. Patients are required to fast for glucose monitoring and refrain from eating or drinking for 2 hours before and 1 hour after each dose.
Patients who show evidence of hyperglycemia during the study should be encouraged to follow a low carbohydrate diet.
Patients should be encouraged to drink at least 20 ounces of fluids a day, especially on days requiring fasting.

Pregnancy
The effects of TAK-228 on human pregnancy and the development of the embryo or fetus are not known. Therefore, female and male patients participating in this study should avoid becoming pregnant or impregnating a partner, respectively.

Women of childbearing potential and men should use effective methods of contraception during and through 90 and 120 days after the last dose of study drug, respectively, 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 highly effective method of contraception and 1 additional (barrier) method, at the same time from the time of ICF signing up to and including 90 days (or longer, as mandated by local labeling [e.g., United Surgical Partners International, summary of product characteristics, etc.]) after the last dose of the study drugs, 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, and postovulation methods] and withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception. Female and male condoms should not be used together.)

Males, even if surgically sterilized (i.e., status postvasectomy), must:

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

11.6 Management of Clinical Events

11.6.1 Management of Hyperglycemia

Based on the TAK-228 clinical trials, most hyperglycemia episodes occurred within the first 60 days after initiation of treatment with TAK-228 and have been grade 1 or 2 and have responded quickly to oral metformin. Hyperglycemia has not been dose-limiting since instituting a standard regimen for early treatment of hyperglycemia.

All patients who develop hyperglycemia during the study should have their glucose closely monitored by study staff. The investigator may choose either to continue close monitoring of patients who develop grade 1 hyperglycemia (FSG > ULN ≤ 160 mg/dL) or, alternatively, consider initiating treatment with an oral hypoglycemic agent, such as metformin. All patients with grade ≥ 2 hyperglycemia (FSG > 160 mg/dL) must be treated aggressively with oral hypoglycemic agents and/or insulin as clinically indicated while continuing on TAK-228. The investigator should consult an endocrinologist, if needed, to aid in optimizing the patient's hyperglycemia treatment plan.

It is recommended that patients be treated initially with a fast-acting insulin sensitizer, such as metformin, at 500 mg p.o. QD and titrate up to a maximum of 1000 mg p.o. twice daily as needed. Concurrent administration of dipeptidyl peptidase-4 inhibitors (e.g., sitagliptin or vildagliptin) and/or insulin should also be considered. Oral sulfonylureas (e.g., glipizide or glyburide) should be used with caution due to the higher hypoglycemia risk. The dose of oral hypoglycemic agents should be adjusted in patients with renal insufficiency. Hyperglycemic patients should also be encouraged to follow a low carbohydrate diet.

If any fasting serum glucose reading performed at the site indicates hyperglycemia (> ULN or ≥ 110 mg/dL), the study staff should first ascertain that the patient was fasting at the time of the blood draw (i.e., nothing by mouth for at least 8 hours prior to blood being obtained).

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 will be given a glucometer to monitor their daily FBG levels at home. The level should be collected daily, predose on dosing days, and at approximately the same time each day.

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

The patient will be instructed to contact the site immediately if the value is abnormal (i.e., ≥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 TAK-228 dose modification for patients with hyperglycemia is provided in Table 8 below.

TABLE 8 MANAGEMENT OF HYPERGLYCEMIA

Grade	Description	Treatment	Dose Modification
1	Fasting blood sugar >ULN to 160 mg/dL	• Continue close monitoring of blood sugar. • Initiate oral hypoglycemic agent.	None
2	>160 to 250 mg/dL	• Initiate oral hypoglycemic agent and/or insulin if not well controlled on oral agent.	None
≥3	>250 mg/dL	• Initiate oral hypoglycemic agent and/or insulin.	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:

- 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.

11.6.2 Management of Hyperlipidemia

Guidance on TAK-228 dose modification for patients with hyperlipidemia is provided in Table 9.

TABLE 9 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	Same as for grade 2.	Same as for grade 3.

Prevention/Prophylaxis:

Life-style modifications, as appropriate (balanced diet, limit alcohol consumption, increase physical activity)

ULN=upper limit of normal.

11.6.3 Management of Oral Mucositis

Guidance on TAK-228 dose modification for patients with oral mucositis is provided in Table 10.

TABLE 10 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 intralesional 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.

11.6.4 Management of Rash

Guidance on TAK-228 dose modification for patients with rash is provided in Table 11.

TABLE 11 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
≥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 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.

11.6.5 Management of Nausea/Vomiting

Guidance on TAK-228 dose modification for patients with nausea and/or vomiting is provided in Table 12.

TABLE 12 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 antiemetic, antinausea, 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.

11.6.6 Management of Cardiac Abnormalities

Management of Patients With Possible Cardiac Instability

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

Management of Patients With Left Ventricular Dysfunction

Guidance on TAK-228 dose modification for patients with left ventricular dysfunction is provided in Table 13.

TABLE 13 MANAGEMENT OF LEFT VENTRICULAR DYSFUNCTION

Grade	Description	Dose Modification
1	Asymptomatic decline in: LVEF >15% from baseline values, OR LVEF >10% to 15% from baseline values and is below institution's LLN.	No change; continue TAK-228 at the same dose and schedule.
≥2	Symptomatic cardiac dysfunction/congestive heart failure.	Discontinue treatment.

LLN=lower limit of normal; LVEF=left ventricular ejection fraction.

Management of Patients with QTc Prolongation

Guidance on TAK-228 dose modification for patients with prolonged QTc interval is provided in Table 14.

TABLE 14 MANAGEMENT OF QTc PROLONGATION

Grade	Description	Treatment	Dose Modification
2	480 msec <QTc <501 msec	Evaluate for other possible causes (e.g., electrolyte disturbance, concomitant medication, etc.).	None; continue TAK-228 at the same dose and schedule.

TABLE 14 MANAGEMENT OF QTc PROLONGATION

Grade	Description	Treatment	Dose Modification
≥3	QTc ≥501 msec	Evaluate for other possible causes (e.g., electrolyte disturbance, concomitant medication[a]). Consider a formal consult by a cardiologist. Notify the study doctor. Additional ECGs may be performed at intervals that the treating physician deems clinically appropriate until repeated QTc measurements fall or are below the threshold interval that triggered the repeat measurement.	Hold TAK-228. The decision whether to reinitiate TAK-228 with or without dose reduction and additional monitoring in those patients who had asymptomatic prolonged QTc ≥501 msec (grade 3) that has reverted to an acceptable interval, have previously tolerated TAK-228, and appear to have benefitted from treatment with either disease control or response, will be agreed to by the investigator on a case-by-case basis Patients who experience persistent symptomatic grade 3 or grade 4 QTc prolongation without another cause should permanently 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/

11.6.7 Management of Other Non-hematologic Toxicities (Including Asthenia, Weakness, and Fatigue)

Guidance on TAK-228 dose modification for patients with other non-hematologic toxicities is provided in Table 15.

TABLE 15 MANAGEMENT OF OTHER NON-HEMATOLOGIC 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, then no adjustment required. • If toxicity becomes intolerable, hold TAK-228 until recovery to ≤grade 1, then reinitiate at the 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 non-hematological 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.

11.6.8 Management of AST/ALT Elevations

Guidance on TAK-228 dose modification for patients with AST/ALT elevations is provided in Table 16.

TABLE 16 MANAGEMENT OF AST/ALT ELEVATIONS

Grade	Description	Treatment	Dose Modification
1	>ULN to 3×ULN	None	None
2	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 (e.g., past medical history, concomitant medications).	None
3	>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 (i.e., Hy's Law).
4	>20×ULN	Same as for grade 2.	Stop TAK-228 and discontinue patient from the study. Permanently discontinue study treatment if in combination with grade 2 total bilirubin elevation when alternative causes cannot be identified (i.e., Hy's Law).
Prevention/Prophylaxis: Ensure proper screening of patients for study participation.			

LFTs=liver function tests; ULN=upper limit of normal.

11.6.9 Management of Non-infectious Pneumonitis

Guidance on TAK-228 dose modification for patients with pneumonitis is provided in Table 17.

TABLE 17 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 3 weeks (21 days), 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.	Interrupt TAK-228 until symptoms resolve to ≤grade 1. • Consider reinitiating TAK-228 at a dose reduced by 1 level . • If toxicity recurs at grade 3, discontinue TAK-228.
4	Life-threatening: Ventilatory support indicated.	Rule out infection and consider treatment with corticosteroids.	Discontinue TAK-228.

11.7 Description of Investigational Agents

TAK-228 will be supplied as capsules for oral administration. The study drug is available in 3 dose strengths, 1 mg, 3 mg, and 5 mg, each containing 1 mg, 3 mg, and 5 mg of TAK-228, respectively, in

addition to the following inactive ingredients: microcrystalline cellulose (solid filler/diluents), magnesium stearate (lubricant), and hard gelatin capsule. All 3 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
- TAK-228 capsules, 5 mg – grey opaque color

Tamoxifen citrate USP is a non-steroidal antiestrogen commercially available in tablet formulation for oral administration. Each tablet contains 10 or 20 mg tamoxifen (equivalent to 15.2 or 30.4 mg tamoxifen citrate, respectively). Tamoxifen is available in generic formulations that may contain the following inactive ingredients depending on the specific generic formulation: croscarmellose sodium, hypromellose, lactose (monohydrate), magnesium stearate, polyethylene glycol 400, povidone, corn starch, and titanium dioxide.

11.8 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. Tamoxifen is commercially available and will be provided through the patient's pharmacy in accordance with all applicable laws and regulations regarding dispensing of a prescription medication.

11.9 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 TAK-228 according to the labeled conditions.

TAK-228 capsules are packaged in 60-cc high-density polyethylene bottles with polypropylene, child-resistant caps and induction seal. For all dose strengths, each bottle contains 30 capsules.

Tamoxifen will be provided through the patient's pharmacy and labeled according to both state and federal laws and regulations.

11.10 Storage, Handling, and Accountability

Upon receipt at the investigative site, the drug should be stored in the original bottles until use and stored at a room temperature of 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 will be stored in the original packaging in a secure area with controlled access. All drug supplies should be used before the retest expiry date. Because TAK-228 is an investigational agent, it should be handled with due care. In case of contact with broken capsules, raising dust should be avoided during the cleanup operation. The product may be harmful if inhaled, ingested, or absorbed through the skin. Gloves and protective clothing should be worn during the cleanup operation. The area should be ventilated and the spill site washed after material pickup 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 (e.g., 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. Tamoxifen should be stored at a controlled room temperature of 20°C to 25°C (68°F to 77°F).

11.11 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.

11.12 Termination of Treatment and/or Study Participation

Patients will be informed that they have the right to withdraw from the study at any time for any reason without prejudice to their medical care. The investigator also has the right to withdraw patients from the study for any of the following reasons:

- AE
- Protocol violation
- Lost to follow-up
- Progressive disease
- Study termination
- Other

At the time of withdrawal, all study procedures outlined for the End of Study visit should be completed. The primary reason for the patient's withdrawal from the study should be recorded in the source documents and CRF.

12. CRITERIA FOR RESPONSE

This study is designed to determine the change in Ki67 and pCR rate after TAK-228 in combination with tamoxifen in patients with ER-positive, HER2-negative breast cancer.

12.1 Response Evaluation Criteria in Solid Tumors (RECIST) 1.1

The clinical tumor response will be assessed at baseline and before surgery using RECIST 1.1. RECIST 1.1 offers a simplified, conservative, extraction of imaging data for wide application in clinical trials. They presume that linear measures are an adequate substitute for 2-dimensional methods and register four response categories.[41]

Target Lesions (Main Tumor)

- 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, using the baseline sum diameters as the reference.
- Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions using the smallest sum while on study (includes the baseline sum) as the reference. In addition to the relative 20% increase, 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 progression)
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, using the smallest sum diameters while on study as the reference.

  Non-Target Lesions (Lymph Nodes)

  CR: Disappearance of all non-target lesions and normalization of tumor marker levels. 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 levels above the normal limits.

- 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 principal investigator.

13. STATISTICAL AND QUANTITATIVE ANALYSES

13.1 Statistical Methods

Based on prior data for Ki67 changes in the tamoxifen only arm of the IMPACT trial, we will assume null and alternative hypotheses of 60% and 80% reduction in Ki67, respectively.[42] Transformations of the geometric mean and 95% CI were employed to obtain mean and standard deviation estimates.

As a measure of safety, toxicity will be monitored during the trial based on a beta-binomial model, assuming a priori that the probability of toxicity p is distributed beta(0.2, 0.8). Study accrual will be suspended and the safety profile of the TAK-228 and tamoxifen combination will be reviewed by the safety monitoring committee if $P_r(p > .20 \mid \text{data}) > 0.85$. This stopping rule yields the following stopping bounds where the numerator represents the number of events needed to suspend accrual and the denominator represents the number treated at that point in the study: 3/6, 4/8, 5/12, 6/16, 7/20, 8/24. An event is defined as progression within 16 weeks. Scenario 1 of the operating characteristics table (see below) indicates that the probability of stopping the study early for progression is very low when progression is uncommon; that is, the study has only a 4.2% chance of stopping early when the true progression rate is only 10%. The study has a 94.3% chance of stopping if the true progression rate is 40% and on average would take only 7 patients to arrive at that conclusion. The stopping rule assumes monitoring begins after 6 patients have been enrolled on study.

Scenario	True P_r(toxicity)	P_r(stop early)	Median # Pts (25%, 75%)
1	0.10	0.042	28 (28, 28)
2	0.20	0.317	28 (15, 28)
3	0.30	0.723	14 (6, 28)
4	0.40	0.943	7 (6, 13)
5	0.50	0.995	6 (6, 7)

13.1.1 Determination of Sample Size

A sample of 25 patients will provide 86% power to detect the hypothesized reduction in Ki67 with 5% alpha based on a two-sided, one sample t-test of mean percent change in Ki67 level, based on prior data for Ki67 changes in the tamoxifen only arm of the IMPACT trial.[42] To account for attrition (estimated 20% screen failure/withdrawal rate) and 10% rate of unmatched pre- versus post-treatment biopsy samples, a sample of 35 patients is proposed for this study.

13.1.2 Randomization and Stratification

Randomization is not employed for this phase II study.

13.1.3 Populations for Analysis

All patients who received at least 1 dose of the study drugs will be included in a descriptive safety analysis. The safety profiles will be assessed through summaries of AEs, SAEs, AEs leading to treatment

discontinuation, and treatment-related death. The safety analysis will report the frequency of all AEs and laboratory abnormalities, as well as the frequency of dose interruptions, dose reductions, and toxicity-related treatment discontinuation. Toxicity rates will be presented using the worst NCI CTCAE version 4.03 grade per patient.

13.1.4 Procedures for Handling Missing, Unused, and Spurious Data

Data quality control will be performed on a regular basis by the research coordinator/research nurse to ensure timely, accurate, and complete patient data collection. Queries will be generated and resolved prior to the generation of interim and final summary reports.

13.1.5 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized for all patients participating in the study using descriptive analysis. Classification of patients as responders versus non-responders will be based on treatment response and changes in associated proliferation markers.

13.1.6 Efficacy Analysis

The single-arm, single-stage, phase II IMPACT clinical trial will be utilized to assess changes in Ki67 levels. Ki67 levels will be log transformed (natural logarithm) to achieve approximately normally distributed data.[42] The difference between the log-transformed values in pre- and post-treatment biopsy samples will be calculated. Based on prior data for Ki67 changes in the tamoxifen arm alone, we will assume null hypothesis and alternative hypotheses of 60% and 80% reduction in Ki67, respectively. Transformations of the geometric mean and 95% CI presented in Dowsett et al.[42] were employed to obtain mean and standard deviation estimates. A sample of 25 patients will provide 86% power to detect the hypothesized reduction in Ki67 with 5% alpha based on a two-sided, one sample t-test of mean percent change in Ki67 level. To account for attrition (estimated 20% screen failure/withdrawal rate) and 10% rate of unmatched pre- versus post-treatment biopsy samples, a sample of 35 patients is proposed in the study.

We assume that the combination will double the pCR rate compared to tamoxifen alone. Assuming a pCR rate of 10% for tamoxifen alone, a one-tailed one sample test for population proportions based on the lower confidence limit of a Wilson's Score interval will achieve 68.6% power at the 0.10 significance level to detect a pCR rate increase due to the addition of TAK-228 to tamoxifen assuming the true pCR rate of the combination is 20%. Assuming a true pCR rate of 20% for the combination, we expect a lower bound for the Wilson Score 90% one-sided confidence interval of 12.4%. The power calculation is based on a simulation of 20,000 replications using the BINOMIAL option of the FREQ procedure of SAS. All statistical analyses will be conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

13.1.7 PK/Biomarkers

Plasma PK of the TAK-228 plus tamoxifen combination will be determined on days 1 and 15. Blood samples for PK analysis will be obtained 1 hour (± 15 minutes) before TAK-228 dosing and 1 hour (± 5 minutes) after TAK-228 dosing. Blood samples (10 mL per sampling timepoint) will be collected and **plasma** batched for future analyses. Banked tumor tissue obtained as part of the patient's standard care and collected biopsy tissues will be evaluated for tissue-based biomarkers including ER; Ki67; mitotic index; apoptosis; levels of S6K1, 4EBP-1, EIF4E, EIF4G, and EIF4A; levels of phosphorylated S6K, p53, p63, and p73 levels; and p73 and p63 gene signatures. These biomarkers will be compared to clinical parameters such as tumor response to help define a biomarker signature associated with p63/p73 and/or PI3K/AKT dependence and NFkB in ER-positive breast cancers and to identify new therapeutic options for ER-positive breast cancer patients. Oncotype DX testing will also be performed on biopsy and surgical

tissue samples.

13.1.8 Safety Analysis

All patients who received at least 1 dose of the study drugs will be included in a descriptive safety analysis. The safety profiles will be assessed through summaries of AEs, SAEs, AEs leading to treatment discontinuation, and treatment-related death. The safety analysis will report the frequency of all AEs and laboratory abnormalities, as well as the frequency of dose interruptions, dose reductions, and treatment discontinuation for toxicity. Toxicity rates will be presented using the worst NCI CTCAE version 4.03 grade per patient.

14. AES

14.1 Definitions

14.1.1 AE Definition

AE is defined as any untoward medical occurrence in a patient or subject administered a pharmaceutical product; the untoward medical occurrence does not necessarily have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not it is related to the medicinal product. This includes any newly occurring event or a previous condition that has increased in severity or frequency since the administration of the study drug. An abnormal laboratory value will not be assessed as an AE unless that value leads to discontinuation or delay in treatment, dose modification, therapeutic intervention, or is considered by the investigator to be a clinically significant change from baseline.

14.1.2 SAE Definition

SAE is defined as any untoward medical occurrence that at any dose:

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

Clarification should be made between a SAE and an AE that is considered severe in intensity (grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term *severe* is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a grade 3 headache). This is NOT the same as *serious*, which is based on patient/event outcome or action criteria described above and usually associated with events that pose a

threat to a patient's life or ability to function. A severe AE (grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm^3 to less than 2000 is considered grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

14.2 Procedures for Reporting SAEs

AEs may be spontaneously identified by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

SAEs must be reported to Takeda Pharmacovigilance (or designee) from the time of consent up to and including 30 days after administration of the last dose 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 Takeda 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).

Since this is an investigator-initiated study, the sponsor-investigator Dr. Jenny Chang is responsible for reporting SAEs to any regulatory agency and to the sponsor-investigator's ethics committee or institutional review board. Regardless of expectedness or causality, all SAEs must also be reported in English to Takeda Pharmacovigilance or designee:

- **Fatal and Life-threatening SAEs:** within 24 hours of the sponsor-investigator's observation or awareness of the event
- **All other serious (non-fatal/non-life-threatening) events:** within 4 calendar days of the sponsor-investigator's observation or awareness of the event

The Sponsor will send all SAE reports to Takeda Pharmacovigilance (or designee) within 24 hours but no later than 4 calendar days as per any agreements.

The SAE report must include at minimum:

- **Event term(s)**
- **Serious criteria**
- **Intensity of the event(s):** Sponsor-investigator's or sub-investigator's determination. Intensity for each SAE, including any lab abnormalities, will be determined using the NCI CTCAE version specified in the protocol, as a guideline, whenever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.
- **Causality of the event(s):** Sponsor-investigator's or sub-investigator's determination of the relationship of the event(s) to study drug administration.

Follow-up information on the SAE may be requested by Millennium Pharmacovigilance (or designee).

In the event that this is a multisite study, the sponsor-investigator is responsible to ensure that the SAE reports are sent to Takeda Pharmacovigilance (or designee) from all sites participating in the study. Sub-investigators must report all SAEs to the sponsor-investigator so that the sponsor-investigator can meet her foregoing reporting obligations to the required regulatory agencies and to Takeda Pharmacovigilance, unless otherwise agreed between the sponsor-investigator and sub-investigator(s).

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).

Houston Methodist Cancer Center at:
hmccsaereports@houstonmethodist.org
Fax #: **713-790-5106**
US and Canada
Toll-Free Fax #: 1-800-963-6290
E-mail: takedaoncocases@cognizant.com
All other countries (Rest of World)
Fax #: 1 202 315 3560
E-mail: takedaoncocases@cognizant.com

Suggested Reporting Form:

- SAE Report Form (a sample will be provided)
- US FDA MedWatch 3500A: http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm
- Any other form deemed appropriate by the sponsor-investigator

14.3 Procedures for Reporting Drug Exposure During Pregnancy and Birth Events

If a patient becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately and permanently discontinue the study drugs. The sponsor-investigator must immediately fax a completed Pregnancy Form to Takeda Pharmacovigilance or designee. The pregnancy must be followed for the final pregnancy outcome (i.e., delivery, still birth, miscarriage) and Takeda 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 Takeda Pharmacovigilance or designee. Every effort should be made to follow the pregnancy for the final pregnancy outcome.

Suggested Pregnancy Reporting Form:
Pregnancy Report Form (a sample will be provided by Takeda Pharmaceuticals)

15. ADMINISTRATIVE REQUIREMENTS

15.1 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 Takeda (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 Takeda 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 or Medication Errors, call

For ADCETRIS or PIPELINE Products:
Phone: 1-844-ONC-TKDA (1-844-662-8532)
Email: GlobalOncologyMedInfo@takeda.com

Fax: 1-800-881-6092, Hours Mon – Fri, 9 a.m. – 7 p.m. ET

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 Takeda Pharmacovigilance.

16. REFERENCES

1. Infante JR, et al, A phase 1, open label, dose escalation study of an investigational oral mammalian target of rapamycin inhibitor TAK-228 (INK128) administered once daily in patients with advanced malignancies. ASCO Abstract 5588.
2. Bachelot T, et al, Randomized Phase II trial of everolimus in combination with tamoxifen in patients with hormone receptor–positive, human epidermal growth factor receptor 2–negative metastatic breast cancer with prior exposure to aromatase inhibitors: a GINECO study J Clin Oncol 2012;30:2718-24.
3. Maiso P, et al. Defining the role of TORC1/2 in multiple myeloma. Blood 2011;118: 6860-70.
4. Ghobrial IM, et al. TAK-228 (INK128), an investigational oral gual TORC1/2 inhibitor, in patients (pts) with relapsed or refractory multiple myeloma (MM), non-Hodgkin's lymphoma (NHL), or Waldenstrom macroglobulinemia (WM): preliminary results from a phase 1 dose-escalation study. ASH 2012, Abstract 4038.
5. ClinicalTrials.gov. Safety and Efficacy Study of TAK-228 in Combination with Exemestane or Fulvestrant in Postmenopausal Women with ER/PR+ Metastatic Breast Cancer. NCT02049957.
6. ClinicalTrials.gov. Phase Ib Study of MLN0128 in Combination with MLN1117 in Adult Patients With Advanced Nonhematologic Malignancies. NCT01899053.
7. Desta Z, et al. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. J Pharmacol Exp Ther 2004;310:1062-75.
8. Wang DY, et al. Identification of estrogen-responsive genes by complementary deoxyribonucleic acid microarray and characterization of a novel early estrogen-induced gene: EEIG1. Mol Endocrinol 2004;18:402-11.
9. Shang Y, et al. "Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription". Cell 2000;103:843-52.
10. Massarweh S, et al.. Tamoxifen resistance in breast tumors is driven by growth factor receptor signaling with repression of classic estrogen receptor genomic function. Cancer Res 2008;68:826-33.
11. Hurtado A, et al. Regulation of ERBB2 by oestrogen receptor-PAX2 determines response to tamoxifen. Nature 2008;456:663-6.
12. "New mechanism predicts tamoxifen response: PAX2 gene implicated in tamoxifen-induced inhibition of ERBB2/HER2-mediated tumor growth". www.modernmedicine.com. 2008-11-13. Retrieved 2008-11-14.
13. Osborne CK, et al. Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer". J. Natl. Cancer Inst 2003;95:353-61.
14. "Study sheds new light on tamoxifen resistance". News. CORDIS News. Retrieved 2008-11-14.
15. Jordan VC. Fourteenth Gaddum Memorial Lecture. A current view of tamoxifen for the treatment and prevention of breast cancer. Br J Pharmacol 1993;110:507-17.
16. "Breast cancer in men". CancerHelp UK. Cancer Research UK. 2007-09-28. Retrieved 2009-03-22.
17. Center for Drug Evaluation and Research (03/08/2005). "Tamoxifen Information: reducing the incidence of breast cancer in women at high risk". U.S. Food and Drug Administration. Archived from the original on June 19, 2007. Retrieved July 3, 2007.
18. National Cancer Institute (2006-04-26). "Study of Tamoxifen and Raloxifene (STAR) Trial". U.S. National Institutes of Health. Retrieved July 3, 2007.
19. University of Pittsburgh. "STAR Study of Tamoxifen and Raloxifen". Archived from the original on June 11, 2007. Retrieved July 3, 2007.
20. Dr. Susan Love (April 22, 2006). "Study Finds New Use for Raloxifene: Reducing Breast Cancer in High-Risk Postmenopausal Women". Retrieved March 19, 2009.
21. "Tamoxifen vs Arimidex as Adjuvant Therapy | Health and Life.
22. Howell A, et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. Lancet 2005;365:60-2.
23. "Arimidex After Two Years of Tamoxifen Reduces Recurrence in Post-Menopausal Women". BreastCancer.org. Archived from the original on February 18, 2008. Retrieved 2008-11-14.

24. Jakesz R, et al.. witching of postmenopausal women with endocrine-responsive early breast cancer to anastrozole after 2 years' adjuvant tamoxifen: combined results of ABCSG trial 8 and ARNO 95 trial". Lancet 2005;366:455-62.
25. USA (2012-10-19). "Tamoxifen treatment of pr... [J Pediatr Endocrinol Metab. 1999 Sep-Oct] - PubMed - NCBI". Ncbi.nlm.nih.gov. Retrieved 2013-01-06.
26. USA (2012-10-19). "Tamoxifen treatment for precocious puberty in McCu... [J Pediatr. 2003] - PubMed - NCBI". Ncbi.nlm.nih.gov. Retrieved 2013-01-06.
27. USA (2012-10-19). "Tamoxifen improved final height p... [J Pediatr Endocrinol Metab. 2006] - PubMed - NCBI". Ncbi.nlm.nih.gov. Retrieved 2013-01-06.
28. USA (2012-12-12). "The use of tamoxifen to improve height potential ... [Pediatrics. 2005] - PubMed - NCBI". Ncbi.nlm.nih.gov. Retrieved 2013-01-06.
29. USA (2012-10-19). "Tamoxifen induces permanent growth arrest through selec... [Bone. 2007] - PubMed - NCBI". Ncbi.nlm.nih.gov. Retrieved 2013-01-06.
30. USA (2012-10-19). "Tamoxifen impairs both longitudinal and cor... [J Bone Miner Res. 2008] - PubMed - NCBI". Ncbi.nlm.nih.gov. Retrieved 2013-01-06.
31. Steiner AZ, et al. Comparison of tamoxifen and clomiphene citrate for ovulation induction: a meta-analysis. Hum. Reprod 2005;20:1511-5.
32. van Bommel EF, et al. Brief communication: tamoxifen therapy for nonmalignant retroperitoneal fibrosis. Ann Intern Med 2006;144:101-6.
33. Sample, Ian (2007-06-13). "Q&A: Chemical castration". Guardian Unlimited. Retrieved 2007-09-10.
34. Chiang GG, et al. Targeting the mTOR signaling network in cancer. Trends Mol Med 2007;13:433-42.
35. Sabatini DM. mTOR and cancer: insights into a complex relationship. Nature Rev Cancer 2006;6:729-34.
36. Genpharm Inc. Tamoxifen full prescribing information. 18 August 2003.
37. Voss M, et al. Phase I study of investigational oral mTORC1/2 inhibitor TAK-228 (formerly MLN0128): expansion phase in patients with renal, endometrial, or bladder cancer. ESMO 2015, Abstract 364.
38. Ghobrial IM, et al. TAK-228 (formerly MLN0128), an investigational oral dual TORC1/2 inhibitor: a phase I dose escalation study in patients with relapsed or refractory multiple myeloma, non-Hodgkin lymphoma, or Waldenstrom's macroglobulinemia. Am J Hematol 2016;91:400-5
39. Beaver JA, et al. The BOLERO-2 trial: the addition of everolimus to exemestane in the treatment of postmenopausal hormone receptor-positive advanced breast cancer. Future Oncol 2012;8:651-7.
40. De Laurentiis M, et al. A meta-analysis on the interaction between HER-2 expression and response to endocrine treatment in advanced breast cancer. Clin Cancer Res 2005;11:4741-8.
41. National Cancer Institute RECIST Guidelines and criteria.
42. Dowsett M, et al. Short-term changes in Ki-67 during neoadjuvant treatment of primary breast cancer with anastrozole or tamoxifen alone or combined correlate with recurrence-free survival. Clin Cancer Res 2005;11(2 Pt 2):951s-8s.

APPENDICES

Appendix A: CTCAE Version 4.03 (dated June-14-2010)

NCI Common Terminology Criteria for Adverse Events (CTCAE) v.4.03 data files and related documents are published here. The most current release files appear in this directory:

Files: Booklet	Content
CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf	Most recent release of core terminology: PDF document, traditional small booklet format.

http://evs.nci.nih.gov/ftp1/CTCAE/About.html

Appendix B: List of Antidepressants and Association with CYP2D6 and Tamoxifen

Class of drugs	Drug	CYP2D6 activity	Tamoxifen Interaction
Selective Serotonin Reuptake Inhibitors	Fluoxetine	Strong inhibitor	Probable
	Paroxetine	Strong Inhibitor	Probable
	Sertraline	Moderate inhibitor	Possible
	Fluvoxamine	Weak inhibitor	Not likely
	Citalopram	Weak inhibitor	Not likely
	Escitalopram	Weak inhibitor	Not likely
Selective Norepinephrine Reuptake Inhibitors	Duloxetine	Moderate inhibitor	Possible
	Venlafaxine	Weak inhibitor	Not likely
	Desvenlafaxine	Weak inhibitor	Note likely
Monoamine Oxidase Inhibitors	Tranylcypromine	Moderate inhibitor	Possible
	Selegiline	Weak inhibitor	Not likely
Tricyclics	Clomipramine	Moderate inhibitor	Possible
	Amitriptyline	Weak inhibitor	Not likely
	Desipramine	Moderate inhibitor	Possible
	Nortryptyline	Weak inhibitor	Not likely
	Imipramine	Moderate inhibitor	Possible
	Doxepin	Major substrate	Not likely
	Trimipramine	Major substrate	Not likely
	Buspirone	Major substrate	Not likely
	Trazodone	Major substrate	Not likely
	Mirtazapine	Weak inhibitor	Not likely
	Bupropion	Strong inhibitor	Probable

Drug interactions assigned documentation levels as outlined by Facts & Comparisons 4.0:

- Certain: proven to occur in studies or recommended by reputable guidelines
- Probable: very likely, but not proven in controlled studies
- Possible: could occur, but data are very limited
- Not likely: no good evidence of an altered clinical effect

Appendix C: Eastern Cooperative Oncology Group Performance Status Scale

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction
1	Symptoms but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work)
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Source: Oken MM, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-55.

Appendix D: New York Heart Association Functional Classifications

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.

Appendix E: List of Relevant Cytochrome P450 Inhibitors and Inducers

Strong CYPA12 Inhibitors		
fluvoxamine	ciprofloxacin	
Moderate CYPA12 Inhibitors		
cimetidine	methoxsalen	
Clinically Significant Enzyme Inducers		
carbamazepine	rifabutin	St. Johns Wort
phenobarbital	rifampin	
phenytoin	rifapentine	

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

Note that these lists are not exhaustive.