TITLE: A Phase II Study of TAK-228 in patients with previously treated metastatic renal cell carcinoma

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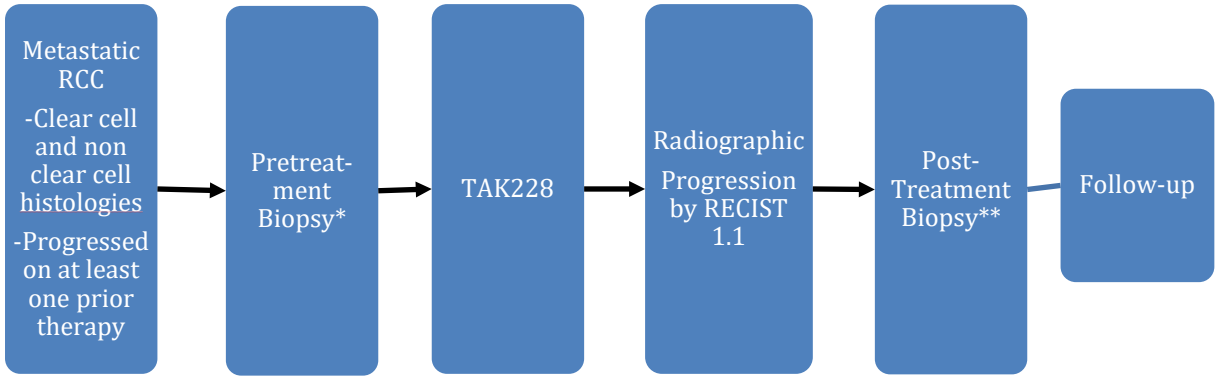
SCHEMA



*Pre-treatment biopsy will be mandatory in subjects who do not have available archival tissue collected within 18 months of enrollment.
**Post-treatment biopsy, if feasible and safe, is mandatory only in subjects who experience a disease response as per RECIST 1.1 criteria followed by subsequent progression.
+One Cycle is 28 Days

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1. STUDY OBJECTIVES AND ENDPOINTS

1.1 Objectives

1.1.1 Primary Objective

Assess the overall response rate (ORR) of TAK-228 in previously treated metastatic Renal Cell Carcinoma (RCC).

1.1.2 Secondary Objectives

1. Evaluate the progression-free survival (PFS) of TAK-228 in previously treated metastatic RCC.
2. Evaluate overall survival (OS) of TAK-228 in previously treated metastatic RCC.
3. Assess toxicity and safety of treatment with TAK-228.

1.1.3 Correlative/Exploratory Objectives

1. Assess the ORR of TAK-228 in subjects with mutations in genes involved in the mTOR pathway.
2. Correlate ORR to TAK-228 to time on prior anti-cancer therapy, specifically prior rapalogs.
3. Correlate ORR to TAK-228 with mutation status in RCC.
4. Examine genetic mechanisms of resistance to TAK-228 in subjects who respond and then progress.

2. BACKGROUND AND RATIONALE

2.1 Renal Cell Carcinoma

Metastatic RCC accounts for 14,000 deaths each year in the U.S. (1). It is a heterogeneous disease divided generally into two major groups: clear-cell RCC (ccRCC) (80%) and non-clear cell RCC (nccRCC). Despite the recent advances in the treatment of metastatic RCC, the median survival remained only 22 months in 2009(2).

The introduction of molecularly targeted therapies has transformed the management of RCC. Since the first approval of sorafenib in December 2005, seven different drugs have been shown to provide clinical benefit for patients with RCC (2-4). Agents targeting the VEGF pathway have emerged as the most robust therapeutic option, and are therefore the initial treatment choice for metastatic RCC. Sunitinib, sorafenib, pazopanib (VEGF receptors tyrosine kinase inhibitors (TKIs)) and bevacizumab (monoclonal antibody against VEGF ligand) are Food and Drug Administration (FDA)-approved in advanced RCC. Another class of targeted agents used in metastatic RCC are allosteric inhibitors of mTOR complex 1 (mTORC1). Everolimus and temsirolimus, both sirolimus analogs (rapalogs), are also FDA-approved for use in the treatment of metastatic RCC based on large randomized phase III trials (3, 4). However, the clinical benefit from mTOR inhibitors is generally modest, with few partial responses (PRs) and very rare complete responses (CRs).

2.2 TAK-228

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

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

The mTORC is an important therapeutic target that is a key intracellular point of convergence for a number of cellular signaling pathways. Inhibiting mTOR may inhibit abnormal cell proliferation, tumor angiogenesis, and abnormal cellular metabolism, thus providing the rationale for mTOR inhibitors as potential agents in the treatment of a number of indications including solid tumor and hematological malignancies, as either monotherapy or in combination with other chemotherapeutic agents. Like rapamycin, several newly approved rapalogs (temsirolimus and everolimus) are specific and allosteric inhibitors of mTORC1, and only partially inhibit

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

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

2.2.1 Pharmacology

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

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

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

2.2.2 Preclinical Studies

Several preclinical studies have showed the superiority of TAK-228 over rapamycin and rapalogs in inhibiting tumor growth in several cancers, such as: breast and pancreatic cancer, glioblastoma, multiple myeloma and B-cell acute lymphoblastic leukemia (B-cell ALL).

TAK-228 reduces p-4EBP1 (phosphorylated-eukaryotic translation initiation factor 4E binding protein 1) and p-S6K1 (phosphorylated-ribosomal S6 kinase 1), reflecting mTORC1 inhibition, as well as p-AKT (also known as protein kinase B) and p-NDRG1 (phosphorylated N-myc downstream-regulated gene 1) reflecting mTORC2 inhibition (5-7). In contrast, rapalogs only decrease phosphorylation of p-S6K1 with variable effects on p-4EBP1, and increases phosporylation of Akt (5, 7, 8). Akt phosphorylation due to rapalog treatment is due to feedback activation of the phosphatidylinositol 3-kinase (PI3K) /Akt pathway, and may contribute to the limited clinical benefit of rapalog mTORC1 inhibitors. Consistent with this model, TAK-228 was found to be effective in cancer cells where the PI3K/Akt pathway has been upregulated, such as HER2 (human epidermal growth factor 2)-positive breast cancer cell lines resistant to HER2 inhibition (transtuzumab and lapatinib) (9). TAK-228 also inhibited cell proliferation in breast cancer cell lines harboring PIK3CA (phosphoinositide-3-kinase-catalytic-alpha), PTEN (phosphatase and tensin homolog), KRAS (Kirsten rat sarcoma viral oncogene homolog), and/or BRAF mutations as well as xenograft models that are both non- VEGF and VEGF-driven (6).

TAK-228 has a cytotoxic effect on several cancer cell lines through the induction of apoptosis, contrasting the cytostatic effect of rapamycin and rapalogs (5, 7). It has also been shown to decrease the invasiveness of prostate cancer cell lines through the inhibition of translation of the genes that play a role in the invasion and metastasis potential, such as: YB1 (Y-box binding protein 1), MTA1 (metastatic associated 1), vimentin and CD44 (cluster of differentiation 44) (5). It also inhibits the VEGF-induced lung metastasis in xenografts models of breast cancer (6). In pancreatic cancer cell lines, TAK-228 also enhanced their radiosensitivity through the inhibition of deoxyribonucleic acid (DNA) repair (10).

In a study directly comparing catalytic inhibitors to allosteric inhibitors of mTOR, TAK-228 caused greater inhibition of mTORC1 signaling, mTORC2 signaling, cell cycle progression and translation in most cell lines compared to rapamycin (11). Furthermore, its growth inhibitory effect was greater in cell lines and *in vivo* models that have either intrinsic or acquired resistance to rapamycin, highlighting its efficacy in resistant settings compared to rapamycin(11).

TAK-228 (MLN0128) has also been shown to have a synergistic effect with several drugs, including: dasatinib in Philadelphia Chromosome-positive B –cell ALL cell lines, lapatinib in HER2-positive breast cancer cell lines, melphalan, doxorubicin, and dexamethasone in multiple myeloma cell lines (7-9).

In RCC, a pre-clinical trial using patient-derived tissue slice graft (TSG) models derived from fresh primary RCC specimens was used to compare the effect of TAK-228 versus temsirolimus. TAK-228 consistently suppressed primary RCC growth up to two months whereas temsirolimus only transiently inhibited the growth of TSGs before resistance developed. In addition, TAK-228 was the only drug to reduce liver metastases lending strong support for the use of dual mTORC1/2 inhibitors in RCC (12).

2.2.3 Clinical Studies

2.2.3.1 Clinical trials:

The pharmacokinetics (PK) and pharmacodynamic (PD) of TAK-228 have been evaluated in two phase I studies of TAK-228 as a single agent in patients with advanced solid malignancies (INK 128-001), or refractory multiple myeloma (MM) and Waldenstorm's macroglobulinemia (WM) (INK 128-002). They are also being evaluated in a phase I study of TAK-228 in combination with paclitaxel in patients with advanced solid tumors.

Table 1: Phase I clinical trials of TAK-228

Study No.	Design	MLN0128 Dose (Schedule)	Evaluable PK Population
INK128-001	Multiple ascending doses in patients with advanced solid malignancies	2, 4, 5, 6, & 7 mg (QD) 7, 10, 15, 20, 30, & 40 mg (QW) 6, 9, 12, 16, & 20 mg (QD×3d QW) 7, 10, & 13 mg (QD×5d QW)	106
INK128-002	Multiple ascending doses in patients with relapsed or refractory multiple myeloma or WM	2, 4, 6, & 7 mg (QD) 9 & 12 mg (QD×3d QW)	39
INK128-003	Multiple ascending doses in combination with paclitaxel in patients with advanced solid malignancies (a)	6, 7, 8, 9, & 10 mg (QD×3d QW) 7 mg (QD×5d QW) 30, 40 mg (QW)	47

Data are preliminary for ongoing studies. Data cutoff date: 09 Dec 2014.

Abbreviations: 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, QW=once weekly, WM=Waldenström macroglobulinemia.

(a) MLN0128 doses were administered in 4-week (28-day) cycles in combination with 80 mg/m^2 paclitaxel (dosed once weekly for 3 weeks [Q3W]).

There are several ongoing phase I and II clinical trials of TAK-228 (13).
TAK-228 is currently investigated in nine phase I clinical trials (one of which is completed), as a single agent and in combination with other drugs (paclitaxel, transtuzumab, MLN1117 (PI3K inhibitor), MLN2480 (A, B and C Raf inhibitor), bevacizumab, Ziv-Aflibercept) in patients with advanced solid tumours, recurrent glioblastoma as well as refractory MM or WM. A phase I study is currently evaluating the effect of a 40 mg single dose of TAK-228 on the electrocardiographic QT/QTc interval in participants with advanced solid tumors. Furthermore, there are 3 phase 1b/2 clinical trials, including one that is assessing the efficacy and the safety of TAK-228 with exemestane or fulvestrant in postmenopausal women with estrogen receptor positive (ER+)/Her2- metastatic breast cancer in patients who progressed on everolimus with exemestane or fulvestrant. The efficacy of TAK-228 is being investigated in 3 phase II clinical trials in patients with metastatic anaplastic thyroid cancer, metastatic castration-resistant prostate cancer as well as advanced squamous lung cancer harboring NFE2L2 (Nuclear Factor, Erythroid 2-like 2) and KEAP1 (Kelch-Like ECH-Associated Protein 1) mutations. A pilot study of TAK-228 given before and after surgery in patients with recurrent glioblastoma is also ongoing.

2.2.3.2 Drug Metabolism and Pharmacokinetics

TAK-228 was rapidly absorbed after PO administration to mice, rats, dogs, and monkeys, with high oral bioavailability. [^{14}C] TAK-228 was rapidly and widely distributed throughout the body in Long-Evans rats; radioactivity was eliminated from most tissues at 48 hours post dose. TAK-228 displayed dose-proportional plasma exposures, a moderate propensity to cross the blood-brain barrier, and was modestly bound (70.5%) to human plasma proteins. TAK-228 distributed mainly to the plasma of human blood. There was no obvious concentration-dependent red blood cell (RBC) distribution of TAK-228 in human blood.

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

M1, the single metabolite (monohydroxylation product) observed in human microsomal incubations, was also observed in rats and monkeys.

Recently completed in vitro metabolism experiments in human hepatocytes using 14C-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.

Especially given clinical exposures observed to date after administration of the highest anticipated therapeutic dose to be used in the clinic in oncology indications (total maximum plasma concentration [C_{max}] of 0.48 μM [free C_{max} of 0.14 μM] at 30 mg once weekly [QW]).

2.2.3.3 Pharmacodynamics

In INK128-001, the pharmacodynamic effect of TAK-228 was measured in surrogate tissue (skin) to explore the downstream effectors of mTOR complex-1 inhibition (pS6 and p4EBP1) and mTOR complex-2 inhibition (pAKT (S473), pNDRG1, and pPRAS40 (proline-rich Akt substrate of 40 kDa)). Preliminary data demonstrate reduction of the levels of pS6, p4EBP1, pNDRG1, and pPRAS40 in skin at TAK-228 doses of ≥4 mg. pAKT (S473) had high variability in the staining rendering the results inconclusive.

2.2.3.4 Updated Manufacturing Process

A new TAK-228 (MLN0128) capsule containing milled active pharmaceutical ingredient (API) is available for new clinical studies in 1 mg, 3 mg and 5 mg strengths. In this study, the 5 mg capsules will be provided.

The milled API, may result in faster absorption profile with possibly higher maximum concentration (Cmax), which could result in a different safety profile compared to the previous unmilled API capsules. Therefore, ongoing studies (C31001, C31002 and , TAK-228 (MLN0128)-1004 –A Phase I, open label study to evaluate the safety, tolerability, and pharmacokinetics of TAK-228 (MLN0128) as a single agent and in combination with paclitaxel in adult patients with advanced non-hematological malignancies-), with the new milled API will determine the recommended phase 2 dose (RP2D) for single agent TAK-228

(MLN0128) (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.

The selected dose of 30 mg TAK-228 QW is based on the findings from 2 studies: Study INK128-001 and Study MLN0128-1004. Study INK128-001was the first-in-human study of TAK-228. This was an open-label study designed to determine the maximum tolerated dose (MTD) and to identify dose-limiting toxicities (DLTs) for oral administration of single-agent unmilled TAK-228, and to characterize the safety and tolerability of escalating doses of TAK-228 in patients with advanced solid tumors. In this study, 116 patients with advanced solid tumors received TAK-228 (2 – 40 mg via 4 dosing schedules: QD [once a day] (31 patients), QDx3 QW [3 days per week] (33 patients), QDx5 QW [5 days per week] (22 patients), and QW [once a week] (30 patients).) in the dose escalation phase. 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%, versus 40 mg QW: 19% and 77%) 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 2 below).

Table 2: 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.

1.3 Rationale

The PI3K/Akt/mTOR pathway is involved in most cancer development, including RCC. The mTOR pathway plays a major role in cell growth, survival and migration. mTOR is the central component of 2 multi-protein complexes: mTORC1 and mTORC2.

mTORC1 is a heterotrimeric protein kinase that consists of the mTOR catalytic subunit and two associated proteins, raptor (regulatory-associated protein of mTOR) and mLST8 (mammalian lethal with SEC13 protein 8, also known as GβL (G-protein beta-subunit like)) (14). Activation of mTORC1 leads to the phosphorylation of S6K1, 4E-BP1, and numerous other protein targets (14) (Fig. 1). S6K1 phosphorylates ribosomal protein S6 and is a key regulator in cell growth.

Furthermore, S6K1 represses the PI3K–Akt pathway by inhibiting IRS1 (insulin receptor substrate 1) and IRS2 expression (14) (Fig. 1). Therefore, mTORC1 inhibition leads to feedback activation of the PI3K/Akt pathway implicated in cell growth (15). Phosphorylation of 4E-BP1 inhibits its binding to eukaryotic initiation factor 4E (eIF4E), which enables the cap-dependent translation of complex mRNAs such as Bcl-2 (B-cell lymphoma 2) and VEGF, thereby increasing cell proliferation, survival and angiogenesis (14).

mTORC2 consists of mTOR and mLST8, as well as rictor (rapamycin-insensitive companion of mTOR) and mSin1 (mammalian stress-activated protein kinase [SAPK]-interacting protein, also known as mitogen-activated-protein-kinase-associated protein 1), that are not part of mTORC1 (14). Activated mTORC2 phosphorylates Akt at Serine (Ser) 473, as well as several other protein targets, leading to enhanced cell survival, proliferation, and cell migration (14) (Fig. 1). Rapamycin and its analogs (rapalogs) inhibit mTORC1 by binding to the rapamycin-binding protein FKBP12, which then binds to mTORC1 (14). Rapamycin has different degrees of inhibition for different substrates of mTORC1. It potently inhibits S6K1 phosphorylation, but has more variable effects on 4EBP1 phosphorylation and cap-dependent translation. In addition, rapalogs lead to feedback activation of Akt - as a result of inhibition of the S6K/IRS-1 feedback loop- decreasing its beneficiary effect as an anticancer drug (11, 15). In PTEN-deficient glioblastoma patients treated with rapamycin, Akt hyperactivation was associated with a shorter time to progression (TTP) (15). Therefore, targeting Akt activation as with mTORC2 inhibition may provide superior clinical benefit.

Rapalogs, such as everolimus and temsirolimus are currently used in the management of mRCC. However, their clinical benefit is limited with very rare CRs and few PRs. Therefore, it is essential to investigate drugs that have a potential increased clinical effect. Novel drugs have been developed to inhibit both mTORC1 and mTORC2. TAK-228 (or INK128) is an ATP-competitive dual mTORC1/2 inhibitor. In contrast to the competitive inhibitors of the mTOR catalytic site, rapamycin and rapalogs (temsirolimus and everolimus) are allosteric inhibitors of mTOR and result in a partial inhibition of the mTOR pathway through the inhibition of mTORC1 complex solely.

Furthermore, competitive dual mTORC1/2 inhibitors such as TAK-228 are thought to be superior to rapalogs in the inhibition of mTOR pathway as well as in anti-tumor activity as it has the advantage of inhibiting the PI3K/Akt pathway. Several pre-clinical studies have demonstrated the superiority of TAK-228 over rapamycin and its analogs, particularly in RCC and in settings of rapamycin resistance (11, 12). Therefore, the evidence suggests that TAK-228 may have benefit for RCC patients who have progressed on rapalogs.

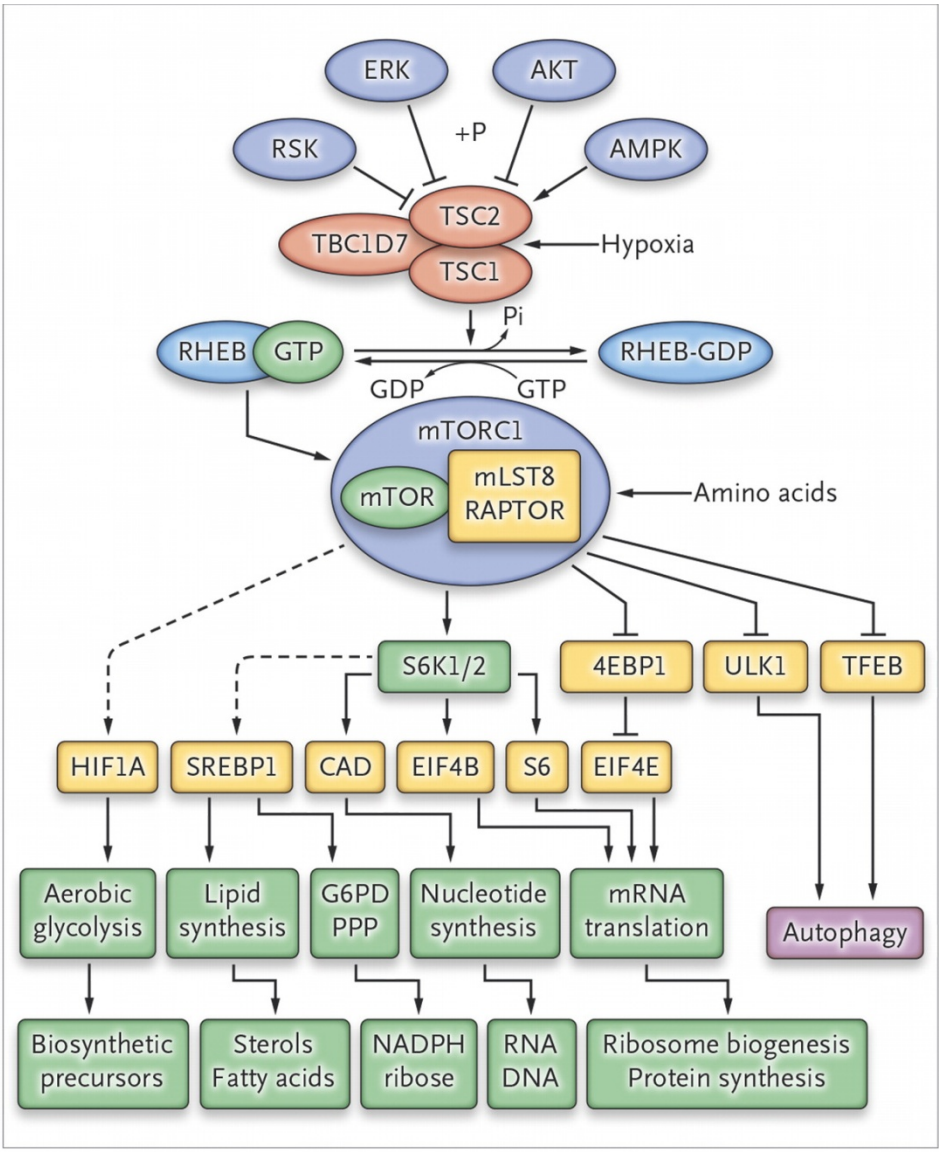


Figure 1. TSC Protein Complex, mTOR Complex 1, and Downstream Effects. The tuberous sclerosis complex (TSC) proteins, which include TSC1, TSC2, and TBC1D7, are negatively regulated by phosphorylation (+P) by AKT, ERK, and RSK, which are all core kinases that are activated during growth signaling. This complex is activated under hypoxia and on phosphorylation by AMPK in response to energy stress and functions as a GTPase-activating protein to negatively regulate RHEB, a Ras family member GTPase. RHEB-GTP activates mechanistic target of rapamycin (mTOR) complex 1 (mTORC1), which is also regulated by intracellular amino acid levels and has multiple direct kinase targets and even more secondary targets of phosphorylation. In some cases, the mechanism of regulation is not completely understood (as indicated by a dashed line). In aggregate, these transcription factors, translation factors, and enzymes lead to biosynthesis of ribosomes and other components that are needed for efficient translation, including ATP and amino acids, as well as lipid precursors, NADPH, nucleotides, RNA, and DNA, to enable an increase in cell size and growth. Arrows indicate stimulatory events, which at times are mediated by phosphorylation; blocked lines indicate

inhibitory effects. Biallelic (complete) loss of TSC1 or TSC2 leads to the loss of negative regulatory effects of the TSC protein complex and constitutive activation of mTORC1. CAD denotes carbamoyl-phosphate synthetase 2, aspartate transcarbamoylase, dihydroorotase; EIF4E eukaryotic translation initiation factor 4E; 4EBP1 eukaryotic translation initiation factor 4E binding protein 1; G6PD glucose-6-phosphate dehydrogenase; HIF1A hypoxia-inducible factor 1 α ; PPP pentose phosphate pathway; SREBP1 sterol regulatory element-binding protein 1; and S6K1/2 ribosomal protein S6 kinase 1 and 2 (16).

1.4 Correlative Studies Background

As described above, TAK-228 is a dual inhibitor of mTORC1/2 signaling, thus it inhibits phosphorylation of their downstream modulators. TAK-228 has been shown to downregulate p-4EBP1,p-S6k, HIF1α (Hypoxia-inducible factor 1α) and MTA1 downstream of mTORC1 as well as AKT, c-Myc and NDRG1 downstream of mTORC2 (5-7, 12, 14).

Acquired secondary mutations in MTOR are one mechanism of resistance to rapalogs, as was demonstrated in a patient with anaplastic thyroid cancer (17). Whole exome sequencing was performed on a tumor sample from a patient who progressed after an impressive 18 months response. A missense mutation in MTOR (F2108L (G>T)) was found only in the biopsy performed at the time of resistance, and not in the pre-treatment tumor. In vitro studies showed that this mutation conferred resistance of mTORC1 to rapalog treatment, but not to mTOR kinase inhibitors, specifically a tool compound Torin1 (11).

Furthermore, it has also been suggested that eIF4E amplification may also be a mechanism of resistance to mTOR inhibitors. eIF4E is an essential component of the eIF4F complex that recruits the mRNA to the ribosome to initiate translation thereby promoting cell growth, survival and angiogenesis. 4E-BPs (4E-binding proteins) are a family of small translational repressors which sequester eIF4E, thereby impeding the assembly of the eIF4F complex. 4E-BP1 is phosphorylated by mTORC1 activation, inhibiting its binding to eIF4E. Alain et al. have shown that a higher eIF4E/4E-BP ratio is predictive of resistance to catalytic mTOR inhibitors (18). Similarly, in a study by Cope et al., amplification of eIF4E has been shown to confer resistance to AZD8005 -a catalytic mTOR inhibitor- with ribonucleic acid interference (RNAi)-mediated knockdown of eIF4E reversing the acquired resistance (19).

Several genes have been found to be significantly mutated in clear cell RCC including HIF1α, HIF1β, PBRM1 and SETD2. Additionally, the cancer genome atlas research network have identified and validated 19 significantly mutated genes (SMGs) in ccRCC with VHL, PBRM1, SETD2, KDM5C, PTEN, BAP1, MTOR and TP53 being the most significant(20). In chromophobe RCC, the SMGs included TP53, PTEN, mTOR, NRAS, TSC1 and TSC2(21). 22% (15/66) of the somatic alterations occurred in the genetic components affecting the mTOR pathway, indicating its role in chromphobe RCC.

3. ELIGIBILITY CRITERIA

3.1 Inclusion Criteria

Subject must meet all of the following applicable inclusion criteria to participate in this study:

1. Age ≥ 18 years.
2. Measurable disease according to RECIST 1.1 within 28 days prior to registration.
3. Documented pathologic diagnosis of RCC. All subtypes eligible including but not limited to clear cell, papillary, chromophobe, collecting duct carcinoma, medullary carcinoma, and unclassified categories. Sarcomatoid and rhabdoid differentiation are allowed.
4. Patients with clear cell histology must have demonstrated: 1) Progression on at least one prior anti-angiogenic agent unless intolerable; AND 2) progression on at least one agent that blocks the PD-1 pathway unless felt by the treating physician to be contraindicated (examples include but are not limited to: patients with autoimmune disease or patients requiring systemic steroids greater than 10 mg/day prednisone or its equivalent) or if they have been discontinued due to toxicity. Prior rapalogues are allowed.
5. Patients with non-clear cell histology must have received at least one prior anti-cancer therapy. Prior rapalogues are allowed.
6. Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 (Appendix A).
7. Left ventricular ejection fraction (LVEF) greater than or equal to the lower limit of normal (LLN) as assessed by either multigated acquisition (MUGA) scan or echocardiogram (ECHO).
8. Must have adequate organ and bone marrow function.

System	Laboratory Value
Hematological	
Absolute Neutrophil Count (ANC)	≥ 1500 cells/μL (without use of G-CSF 4 weeks prior to enrollment)
Hemoglobin (Hgb)	≥ 9 g/dL (transfusions allowed)
Platelets (Plts)	≥ 100 k/mm^3
Renal	
Calculated creatinine clearance Cockcroft-Gault formula will be used to calculate creatinine clearance	≥ 30 mL/min
Urinalysis	For patients with 2+ proteinuria on urinalysis, 24 hour urine collection should be obtained. 24 hour urine protein should be < 2 grams.
Hepatic	

Bilirubin	≤ 1.5 × upper limit of normal (ULN). For subjects with Gilbert's disease ≤ 3.0 mg/dL
Aspartate aminotransferase (AST)	≤ 2.5 × ULN; ≤ 5 x ULN if liver metastases are present
Alanine aminotransferase (ALT)	≤ 2.5 × ULN; ≤ 5 x ULN if liver metastases are present
Metabolic	
Glycosylated hemoglobin (HbA1c)	< 7.0%,
Fasting serum glucose	≤ 130 mg/dL
Fasting triglycerides	≤ 300 mg/dL

9. Recovery to baseline or ≤ grade 1 Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 from toxicities related to any prior treatment, unless adverse events are clinically non-significant and/or stable on supportive therapy.
10. Capable of understanding and complying with the protocol requirements and has signed the informed consent document. 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 subject at any time without prejudice to future medical care.
11. Submission of formalin-fixed, paraffin-embedded (FFPE) archival tumor specimens from the previous 18 months, if available. If not available, a fresh tumor biopsy prior to treatment initiation is MANDATORY unless determined medically unsafe or not feasible by the site investigator.
    - The archival specimen must contain adequate viable tumor tissue.
    - Specimens may consist of a tissue block (preferred and should contain the highest grade of tumor) or a recommended minimum of 20 unstained serial sections. Fine-needle aspiration, brushings, cell pellet from pleural effusion, bone marrow aspirate/biopsy are not acceptable.
    - Distant metastases specimens are preferred but if not available primary nephrectomy specimens are acceptable.
12. Subjects who experience a disease response per RECIST 1.1 criteria followed by subsequent progression will be required to have a post-treatment biopsy if feasible and safe.
13. Sexually active subjects and their partners must agree to use medically accepted methods of contraception.
    - For women:
        - Postmenopausal for at least 1 year before the screening visit, OR
        - Surgically sterile, OR
        - Agree to practice 1 effective method of contraception and 1 additional effective (barrier) method at the same time, from the time of signing the informed consent through 90 days (or longer, as mandated by local labeling [eg. USPI, SmPC, etc.] after the last dose of study drug, OR
        - Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the subject (Periodic abstinence [e.g, calendar, ovulation, symptothermal, postovulation methods] and withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception. Female and male condoms should not be used together.)

- For men:
  - Even if surgically sterilized (ie, status post-vasectomy), they must agree to practice highly effective barrier contraception during the entire study treatment period and through 120 days after the last dose of study drug, OR
  - Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the subject (Periodic abstinence [e.g, calendar, ovulation, symptothermal, postovulation methods for the female partner] and withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception. Female and male condoms should not be used together.)
  - Agree not to donate sperm during the course of this study or 120 days after receiving their last dose of study drug.

3.2 Exclusion criteria

1. Subjects with a history of deep vein thrombosis (DVT) or pulmonary embolism (PE) within 6 months of study treatment initiation.
2. Receipt of any type of small molecular kinase inhibitor (including investigational kinase inhibitors) within 2 weeks of enrollment or receipt of any anti-cancer therapy (including investigational therapy, monoclonal antibodies, cytokine therapy) within 3 weeks of enrollment.
3. Treatment with any investigational products within 3 weeks before the first dose of study drug.
4. Radiation therapy for bone metastases within 2 weeks, other external radiation therapy within 4 weeks of enrollment.
5. Received prior hemibody external radiotherapy.
6. Known brain metastases or cranial epidural disease unless adequately treated with radiotherapy, radiosurgery, or surgery and stable for at least 4 weeks prior to enrollment as documented by magnetic resonance imaging (MRI) or computed tomography (CT) imaging. Treated brain metastases are defined as having no ongoing requirement for steroids and no evidence of progression or hemorrhage after treatment for at least 4 weeks prior to enrollment as documented by MRI or CT imaging.
7. Imminent or established spinal cord compression based on clinical and/or imaging. In subjects with untreated imminent or established spinal cord compression, treatment with standard of care as clinically indicated should be completed at least 4 weeks before enrollment.
8. The subject has a history of any of the following within the last 6 months before administration of the first dose of the drug:
   - Ischemic myocardial event, including angina requiring therapy and artery revascularization procedures
   - Ischemic cerebrovascular event, including transient ischemic attack and artery revascularization procedures

- Requirement for inotropic support (excluding digoxin) or serious (uncontrolled) cardiac arrhythmia (including atrial flutter/fibrillation, ventricular fibrillation or ventricular tachycardia)
- Placement of a pacemaker for control of rhythm
- New York Heart Association (NYHA) Class III or IV heart failure
- Significant active cardiovascular or pulmonary disease including:
    - Uncontrolled hypertension defined as sustained BP >160 mm Hg systolic or > 95 mm Hg diastolic despite optimal antihypertensive treatment.
    - Pulmonary hypertension
    - Uncontrolled asthma or O2 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
    - History of arrhythmia requiring an implantable cardiac defibrillator
    - Medically significant (symptomatic) bradycardia

9. Poorly controlled diabetes mellitus defined as glycosylated hemoglobin (HbA1c) > 7%; subjects with a history of transient glucose intolerance due to corticosteroid administration may be enrolled in this study if all other inclusion/exclusion criteria are met
10. Active gastrointestinal (GI) disorders including those associated with a high risk of perforation or fistula formation. Subjects with enteric stomata (such as ileostomy, colostomy) are also excluded:
    - Tumors invading the GI-tract, active peptic ulcer disease, inflammatory bowel disease, diverticulitis, cholecystitis, symptomatic cholangitis or appendicitis, acute pancreatitis or acute obstruction of the pancreatic or biliary duct, or gastric outlet obstruction.
    - Abdominal fistula, gastrointestinal perforation, bowel obstruction, or intraabdominal abscess within 12 weeks before enrollment. **NOTE**: Complete healing of an intra-abdominal abscess must be confirmed before enrollment.
11. Clinically significant hematemesis or hemoptysis of > 0.5 teaspoon (2.5 ml) of red blood, or other history of significant bleeding (such as pulmonary hemorrhage) within 4 weeks of enrollment.
12. Other clinically significant disorders such as:
    - Known active infection requiring systemic treatment, infection with human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS)-related illness, chronic hepatitis B or known or suspected active hepatitis C infection.
    - Serious non-healing wound or ulcer.
    - Malabsorption syndrome.
    - Symptomatic hypothyroidism.
    - Moderate to severe hepatic impairment (Child-Pugh B or C).
    - Requirement for hemodialysis or peritoneal dialysis.
    - History of solid organ transplantation.

13. Major surgery (such as GI surgery) within 6 weeks of enrollment. However, subjects who have had a nephrectomy may be enrolled 4 weeks after surgery, providing there are no wound-healing complications. Subjects with clinically relevant ongoing complications from prior surgery are not eligible. The following are not considered to be major procedures: Thoracentesis, paracentesis, port placement, laparoscopy, thoracoscopy, bronchoscopy, endoscopic ultrasonographic procedures, mediastinoscopy, skin biopsies, incisional biopsies, imaging-guided biopsy for diagnostic purposes, and routine dental procedures.
14. QTcF (Fridericia formula for the QT interval correction for the heart rate) > 480 msec within 4 weeks of enrollment. If the initial QTcF is found to be > 480 msec, two additional electrocardiograms (EKGs) separated by at least 3 minutes should be performed. If the average of these three consecutive results for QTcF is ≤ 480 msec, the subject meets eligibility in this regard. Subjects with history of congenital long QT syndrome, or torsades de pointes, are not allowed.
15. Pregnant or lactating females.
16. Inability to swallow tablets or capsules.
17. Previously identified allergy or hypersensitivity to components of the study treatment formulations.
18. Malignancies other than RCC within 5 years of first study treatment with the exception of those with negligible risk of metastases or death (carcinoma in situ of the cervix, basal or squamous cell skin cancer, localized prostate cancer, ductal carcinoma in situ of the breast, non-muscle invasive urothelial carcinoma).
19. Any serious medical or psychiatric illness that could, in the site investigator's opinion, potentially interfere with the completion of treatment according to this protocol.
20. 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 study drug.

3.3 Inclusion of Women and Minorities

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

4. SUBJECT REGISTRATION

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

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

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at the DFCI by the Project Manager. All sites should call the Project Manager to verify treatment availability.

Following registration, participants should begin protocol therapy within 5 days. Issues that would cause treatment delays should be discussed with the Overall PI. If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. The Project Manager should be notified of cancellations as soon as possible.

4.4 Registration Process for Other Investigative Sites

To register a participant, the following documents should be completed by the research nurse or data manager and emailed/faxed to the Project Manager:

- Informed consent
- HIPAA authorization form (if not included in consent form).
- Medical History
- Diagnosis and staging assessment
- Physical Examination including review of medications (over-the-counter or prescribed) and side effects the subject is experiencing
- Vital signs (including temperature, heart rate, blood pressure, weight, height and oxygen saturation) and ECOG performance status
- ECG
- Laboratory Testing
- Documentation of at least one prior anti-cancer therapy
- Eligibility Checklist

The research nurse or data manager at the participating site will then e-mail the Project Manager to verify eligibility. To complete the registration process, the Project Manager will follow DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) and register the participant on the protocol. The Project

Manager will fax or e-mail the participant study number, and if applicable the dose treatment level, to the participating site. The Project Manager will also call the research nurse or data manager at the participating site and verbally confirm registration.

5. TREATMENT PLAN

5.1 Pre-medication and Hydration

Prophylactic use of anti-emetic, anti-nausea, and anti-diarrheal medications are encouraged and may be used prior to first dose of TAK-228, and as needed throughout the study prior to each dosing and as clinically indicated per standard practice. When selecting an anti-emetic agent, drugs that do not have an effect on the QT interval are preferred. Subjects should be encouraged to drink at least 18-24 oz of liquids a day.

5.1.1 Antiemetic Recommendations: These guidelines are strongly recommended to prevent treatment associated nausea but will not constitute deviations if not followed.

Cycle 1 Day 1: IV Palonosetron (Aloxi) 0.25 mg infusion administered in clinic prior to first TAK-228 administration (dosing of study drug may be done outside of clinic).

- Recommend prescribing prochlorperazine 10 mg orally as needed every 6 hours and lorazepam 0.5 mg as needed every 6 hours for nausea following study drug administration. Encourage the patient to alternate between the two anti-emetics if nausea persists.

Cycle 1 Day 8 (and subsequent dosing days): If first dose of TAK-228 was tolerated well with IV palonosetron and as needed anti-emetics, subsequent dosing will include only oral antiemetic prophylaxis prior to TAK-228 dosing.

- Recommend prescribing ondansetron 8mg and instruct patient to take ondanesetron orally at least 30-60 minutes prior to TAK-228 dosing.
- If nausea after TAK-228 dosing persists, recommend providing patient with prochlorperazine 10 mg every 6 hours and lorazepam 0.5 mg every 4-6 hours as needed. Alternating between the two anti-emetics is recommended if nausea persists.

5.2 TAK-228 Administration

Eligible subjects will be registered and will receive treatment with TAK-228 at a dose of 30 mg by mouth weekly on Day 1, Day 8, Day 15 and Day 22. Each cycle will be 4 weeks (28 days) in duration.

TAK-228 capsules should not be opened, dissolved or crushed. TAK-228 capsules should be swallowed whole on an empty stomach. Subjects 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. If the subject chews or sucks the capsules by error, the subject should drink a large glass

of water (~8 oz). Direct contact with the powder in TAK-228 capsules with skin or mucous membranes should be avoided. If such contact occurs, the subject should wash thoroughly with water.

Subjects should be instructed to take their study medication at approximately the same time on each scheduled dosing day and not to take more than the prescribed dose at any time. If a subject does not take his or her TAK-228 dose within the time frame specified (± 24 hours of the QW scheduled dosing time), then the dose should be skipped and considered a missed dose. Subjects 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 subject repeat a dose or double-up doses. The subject will be asked to bring their diary and pill bottle to their clinic visits.

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

TAK-228 Administration

Drug	Dose	Route[1]	Schedule	Cycle Length[2]
TAK-228	30 mg (six 5 mg capsules)	Orally	Day 1, Day 8, Day 15 and Day 22	4 weeks/28 days)
[1] Capsules should be taken whole with water. No food should be consumed within 2 hours before and one hour after the dose. Capsules should not be chewed, crushed or dissolved. If vomited, the capsules should not be retaken. [2] A window of ± 3 days may be applied to all study visits to accommodate observed holidays, inclement weather, scheduling conflicts etc. Date and time of each drug administration should be clearly documented in subject's chart and electronic case report forms (eCRFs).				

5.2.1 Prior to Cycle 1 Day 1, the following parameters must be met:

- ANC ≥ 1500/mm^3.
- Platelet count ≥ 100,000/mm^3.
- Hemoglobin ≥ 9 g/dL (transfusions allowed).

5.2.2 Subsequent cycles

No specific parameters to be met for subsequent cycles.

5.3 Subject Evaluations

Subjects will be evaluated clinically every week for Cycle 1, every 2 weeks for Cycles 2-3, and then every 4 weeks for subsequent cycles. On the days that a subject is seen in clinic, subjects are not required to physically dose in clinic. There is no in-clinic dosing requirement.

Subjects will undergo disease assessments with imaging every 8 weeks relative to the C1D1 visit and continue on study therapy until radiographic progression, unacceptable toxicity or withdrawal.

Subjects that do not have archived tissue from the previous 18 months available will undergo one mandatory biopsy at screening. The pretreatment biopsy must occur within 28-days prior to initiation of therapy. In subjects who have an objective disease response as per RECIST version 1.1 and then subsequently progress, a second biopsy at time of progression will be mandatory if feasible and safe. Subjects should continue study treatment until the day prior to the progression biopsy. Progression biopsy must occur within 28-days of determination of disease progression.

5.4 Concomitant Medications

All prescription and over-the-counter medications, including influenza vaccines, taken by a subject within 30 days before the first study drug administration will be recorded on the designated CRF.

5.4.1 Allowed Concomitant Medications

- Histamine H2 receptor antagonists may be allowed, if needed provided that the histamine H2 receptor antagonist is not taken within 12 hours before and within 6 hours after study drug administration. Subjects receiving histamine H2 receptor antagonists before enrollment must stop using these medications for at least 24 hours before their first dose of study drug. Examples of histamine H2 receptor antagonists include ranitidine, famotidine, and nizatidine. Cimetidine, a moderate cytochrome P450 (CYP)1A2 inhibitor, is not recommended as a first choice H2 receptor antagonist.
- Low molecular weight heparin (LMWH) is allowed
- Palliative XRT to pre-existing lesion is allowed
- Other medications considered necessary for the subject's safety and well-being may be given at the discretion of the site investigator. Any concomitant medications added or discontinued during the study should be recorded on the Case Report Forms (CRF).
- Neutralizing antacid preparations (acid neutralizers) and calcium supplements are not permitted during Cycle 1 on study drug administration days, but may be taken as needed on non-TAK-228 administration days. However, for all other cycles of the study administration of neutralizing antacids and calcium preparations is permitted except from 4 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

5.4.2 Prohibited Concomitant Medications

The following medications/therapies are prohibited during the study:

- Other investigational agents or mTOR inhibitors.
- Other anticancer therapies including chemotherapy, immunotherapy, radioimmunotherapy, targeted agents, radiation or surgery (subjects can have palliative radiation or surgery in the study for pre-existing lesions).

- Systemic corticosteroids (either IV or oral steroids, excluding inhalers or intraarticular, intraocular steroids) unless necessary for treatment of TAK-228 related AE (such as, rash).
- Anti-epileptic drugs for subjects with treated brain metastasis.
- Concomitant administration of any PPI is not permitted during the study. Subjects receiving PPI therapy before enrollment must stop using the PPI for 7 days before their first dose of study drugs. Examples of PPIs include omeprazole, esomeprazole, pantoprazole, lansoprazole, and rabeprazole.
- Strong CYP1A2 inhibitors and CYP inducers should be administered with caution, at the discretion of the investigator (see Appendix C). Alternative treatments, if available, should be considered.
- Use of concomitant medications that are known to prolong the QTc interval is strongly discouraged. If use is medically indicated, caution is advised with increased EKG for QTc monitoring.

5.4.3 Supportive Care

Subjects should receive full supportive care, including transfusions of blood and blood products, antibiotics, anti-emetics, etc., when appropriate. Subjects who require therapeutic anticoagulation with low molecular weight heparin (e.g., enoxaparin and tinzaparin) at study entry will be eligible for enrollment. In addition, subjects requiring therapeutic anticoagulation with these agents during study participation will be allowed to remain on study therapy.

5.5 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- Investigator determines a change of therapy would be in the best interest of the subject
- Female subject becomes pregnant
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator
- Study Termination

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

An ODQ Treatment Ended/Off Study Form will be filled out when a participant is removed from protocol therapy. This form can be found on the DF/HCC website at http://www.dfhcc.harvard.edu/research/clinical-research-support/document-library-forms-sops-etc/.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, [redacted]

5.6 Duration of Follow Up

Subjects will be followed for safety for 90 days following the last dose of study treatment or until receipt of another anticancer therapy, whichever comes first. Subjects who have an ongoing study treatment-related AE upon study completion or at discontinuation from the study will be followed until the event has resolved to baseline grade, the event is assessed by the site investigator as stable, new anticancer treatment is initiated, the subject is lost to follow-up, the subject withdraws consent, or until it has been determined that study treatment or participation is not the cause of the AE.

After progression/treatment discontinuation, subjects will be followed for survival and receipt of next line therapies every 6 months until death or 2 years after treatment discontinuation Follow-up will be via phone calls and through review of medical records.

5.7 Criteria for Taking a Participant Off Study

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

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

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

For Decentralized Subject Registrations, the research team updates the relevant Off Treatment/Off Study information in OnCore.

5.8 Replacement

A subject who discontinues study participation prematurely for any reason is defined as a "dropout" if the subject has already been assigned to treatment or administered at least one dose of the study drug. Subjects who have dropped out will not be replaced.

6. TOXICITIES AND DOSE DELAYS/DOSE MODIFICATIONS

Measures will be taken to ensure the safety of subjects participating in this trial, including the use of stringent inclusion and exclusion criteria and close monitoring. Eligibility criteria were

selected to guard the safety of subjects in this trial. Safety will be evaluated in this study through the monitoring of all serious and non-serious AEs, defined and graded according to CTCAE v4. Subjects will be assessed for safety (including laboratory values). General safety assessments will include serial interval histories, physical examinations, and specific laboratory studies, including serum chemistries and blood counts all serious adverse events (SAEs) and protocol defined events of special interest will be reported in an expedited fashion. In addition, the site investigators will review and evaluate observed AEs on a regular basis.

The descriptions and grading scales found in the revised CTCAE v4 will be utilized for dose delays. A copy of the CTCAE v4 can be downloaded from the Cancer Therapy Evaluation Program (CTEP) website
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.1 TAK-228 Dose Modifications

TAK-228 administration should be withheld for treatment-related adverse events 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 2 weeks of interrupting treatment, then the subject may resume study treatment at a dose reduced by 1 level (see Table below). If study drug administration is delayed for more than 4 weeks (28 consecutive days) due to non-resolving study drug-related grade 3 toxicity despite supportive treatment per standard clinical practice, or more than 3 dose reductions of study drug are required in a subject, then study drug treatment should be stopped, the subject discontinued from the study, and the EOT visit completed within 30 days (±7) after the last dose of study drug.

If study drug administration is delayed for more than 6 weeks (42 consecutive days) due to non-treatment related adverse events, then study drug treatment should be discontinued, and EOT visit completed within 30 days (± 7 days) after the last dose of study drug

If study drug is delayed for either less than 4 weeks (28 consecutive days) due to non-resolving study drug-related grade 3 toxicity or less than 6 weeks (42 consecutive days) due to non-treatment related adverse events, subjects should skip the dosing cycles missed and when if treatment is resume, this should be at the next scheduled dosing cycle.

6.2 Dose Levels for Dose Reductions

Table 4: Dose Modifications for Single-Agent TAK-228

Dose Level	Dose Regimen	TAK-228 Capsules: Number and Strength
Starting Dose Level	30 mg QW	Six 5-mg capsules
–1	20 mg QW	Four 5-mg capsules
–2	15 mg QW	Three 5-mg capsule
–3	10 mg QW	Two 5-mg capsules

QW=once weekly.

It is not mandatory to hold treatment in the absence of optimal supportive care for toxicities of nausea and diarrhea. Optimal supportive care is defined as an anti-emetic regimen that employs a 5-HT3 antagonist. If nausea and diarrhea persist despite optimal supportive care, treatment should be held until the subject returns to baseline.

In the case that a patient experiences persistent grade 1 or 2 toxicity, treatment with TAK-228 may be withheld at the discretion of the treating investigator. Once toxicity resolves in the case of grade 1 toxicity or resolves or at least improves to grade 1 in the case of grade 2 toxicity, treatment with TAK-228 may be resumed at the current dose level. If persistent grade 1 or 2 toxicity recurs, treatment with TAK-228 can again be held withheld at the discretion of the treating investigator. Once toxicity resolves in the case of grade 1 toxicity or resolves or at least improves to grade 1 in the case of grade 2 toxicity, treatment with TAK-228 may be resumed but at a reduced dose by one level.

6.3 Management of Clinical Events

6.3.1 Management of Hyperglycemia

Guidance for TAK-228 dose management in the event of hyperglycemia is provided in the table below.

Table 5: Management of Hyperglycemia

Grade	Description	Treatment	TAK-228 Dose Modification
1	Fasting blood sugar > ULN–160 mg/dL	Continue close monitoring of blood sugars. Consider initiation of oral hypoglycemic agent and/or insulin if not well controlled on oral agent.	None.
2	Fasting blood sugar > 160–250 mg/dL	Initiate oral hypoglycemic agent and/or insulin if not well controlled on oral agent.	None.
≥3	Fasting blood sugar > 250 mg/dL	Initiate oral hypoglycemic agent and/or insulin.	Hold drugs until ≤ Grade 2. Resume TAK-228 based on timing of recovery: ≤ 1 week: resume at same dose and schedule; >1 but ≤2 weeks: reduce TAK-228 by 1 dose level >2 weeks: discontinue subject from study
Prevention/Prophylaxis • Follow fasting serum glucose levels during clinic visits. • Monitor home glucometer test results. • Check HbA1c levels every 3 months during therapy.			

- Life-style modifications, as appropriate (balanced diet, limit alcohol consumption, increase physical activity).
- Most episodes of Grade 1 and 2 hyperglycemia respond quickly to oral metformin.
- Early initiation of therapy is recommended to prevent higher grade hyperglycemia.
- Fasting blood glucose levels ≥ 150 mg/dL by glucometer should be followed by closer monitoring of serum glucose and possible intervention.

Abbreviations: dL = deciliters; mg = milligrams; ULN = upper limit of normal.

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

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

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

In-Home Daily Fasting Glucose Monitoring

In addition to obtaining fasting glucose levels at the clinic visits as outlined in the Schedule of Events, all subjects receiving TAK-228 will be given a glucometer to monitor their daily FBG levels at home. The level should be collected daily, predose on dosing days, and at approximately the same time each day.

Prior to initiating treatment, the subject will be provided an in-home glucometer. Subjects 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. Subjects 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. Site investigators will be responsible for reviewing the home glucose monitoring logs for hyperglycemia.

The subject will be instructed to contact the site immediately if the value is abnormal (ie, ≥150 mg/dL) for further instructions on the management of their hyperglycemia. Hyperglycemia observed during home glucose monitoring should be confirmed in the clinic. If no irregularities in the fasting blood glucose level are observed during a minimum of 2 consecutive months, then the frequency of in-home fasting blood glucose testing can be reduced to a minimum frequency of once weekly, depending on the site investigator's judgment and approval. Subjects will continue to notify the site investigator of fasting blood glucose levels that exceed 150 mg/dL and, if blood glucose levels are not well controlled, or if the subject requires either oral hypoglycemic agents or insulin to control blood glucose levels, then the frequency of in-home testing of FBG levels will be reinstated to daily.

6.3.2 Management of Noninfectious Pneumonitis

Guidance for TAK-228 dose management in the event of noninfectious pneumonitis is shown in the table below.

Table 6: 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 ADLs	Rule out infection and consider treatment with corticosteroids until symptoms improve to ≤ Grade 1.	Interrupt TAK-228 treatment: When symptoms ≤ Grade 1, re-initiate TAK-228 treatment at a dose reduction Discontinue TAK-228 treatment if failure to recover within 4 weeks.
3	Symptomatic: Interfering with ADLs; Requires administration of O_2	Rule out infection and consider treatment with corticosteroids until symptoms improve to ≤ Grade 1.	Interrupt TAK-228 treatment until symptoms resolve to ≤ Grade 1. Consider re-initiating TAK-228 treatment at a dose reduction If toxicity recurs at Grade 3, discontinue TAK-228 treatment. .
4	Life-threatening: Ventilatory support indicated	Rule out infection and consider treatment with corticosteroids.	Discontinue TAK-228 treatment.

Abbreviations: ADL = activities of daily living; O_2 = oxygen gas.

6.3.3 Management of Hyperlipidemia

Guidance for TAK-228 dose management in the event of hyperlipidemia is shown in the table below.

Table 7: Management of Hyperlipidemia

Grade	Description	Treatment	TAK-228 Dose Modification
1	Cholesterol: > ULN - 300 mg/dL Triglycerides: > 150 - 300 mg/dL	None.	None.
2	Cholesterol: > 300 – 400 mg/dL Triglycerides: > 300 - 500 mg/dL	Treat hyperlipidemia according to standard guidelines. Triglycerides ≥ 500 mg/dl should be treated urgently due to risk of pancreatitis.	Maintain dose if tolerable. If toxicity becomes intolerable, interrupt TAK-228 dosing until recovery to ≤ Grade 1. Reinitiate at same dose.
3	Cholesterol: > 400 - 500 mg/dL Triglycerides: > 500 - 1000 mg/dL	Same as for Grade 2.	Hold dose until recovery to ≤ Grade 1, then restart at a dose reduction
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 consumption of alcoholic beverages, increase physical activity).			

Abbreviations: dL = deciliters; mg = milligrams; ULN = upper limit of normal.

6.3.4 Management of Oral Mucositis

Guidance for TAK-228 dose management in the event of oral mucositis is provided in the table below.

Table 8: Management of Oral Mucositis

Grade	Description	Treatment	TAK-228 Dose Modification
1	Asymptomatic or mild symptoms	Non-alcoholic mouth wash or 0.9% salt water rinse; Consider topical corticosteroids at earliest signs of mucositis.	None.
2	Moderate pain, not interfering with oral intake Modified diet indicated	Topical analgesic mouth treatments; Topical corticosteroids; Initiate antiviral or antifungal therapy, if indicated.	Maintain dose if tolerable. If toxicity becomes intolerable, interrupt TAK-228 dosing until recovery to ≤ Grade 1. Reinitiate at same dose.
3	Severe pain, interfering with oral intake	Same as for Grade 2; Consider intra-lesional corticosteroids.	Hold dose until recovery to ≤ Grade 1, then restart at a dose reduction

4	Life-threatening consequences	Same as for Grade 2. Consider intra-lesional corticosteroids.	Discontinue treatment.

Prevention/Prophylaxis

- Consider initiation of a non- alcoholic mouth wash or 0.9% salt water rinses 4-6 times daily with start of therapy before signs of mucositis develop.
- Avoid using agents containing hydrogen peroxide, iodine, and thyme derivatives in management of stomatitis as they may worsen mouth ulcers.

6.3.5 Management of Rash

Guidance for TAK-228 dose adjustment for the event of rash is provided in the table below

Table 9: Management of Rash

Grade	Description	Treatment	TAK-228 Dose Modification
≤ 2	Macules/papules covering ≤ 30% body surface area with or without symptoms	Consider treatment with topical steroid cream/ointment and/or oral anti-histamines or antibiotics.	None.
≥ 3	Macules/papules covering > 30% body surface area with or without symptoms	Consider treatment with topical steroid cream/ointment, oral antihistamines, oral antibiotics, and/or pulsed steroids.	Hold until ≤ Grade 2; Resume TAK-228 based on timing of recovery and severity of symptoms: ≤ 3 weeks: reduce dose by 1 dose level; > 3 weeks and severely symptomatic: stop TAK-228 and discontinue subject from the study.

Subjects who develop Grade 4 rash should permanently discontinue study treatment, unless they derive clinical benefit as determined by the site investigator, 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 and/or 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 requiring with intravenous (IV) antibiotics indicated; life threatening consequences (NCI CTCAE Version 4.03, effective date 14 June 2010).

Prevention/Prophylaxis:

Rash should be managed aggressively. The site 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.

6.3.6 Management of Nausea and/or Vomiting

Guidance for TAK-228 dose adjustment for the event of nausea and/or vomiting is provided in the table below.

Table 10: Management of Nausea and/or Vomiting

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

Abbreviations: IV = intravenous; IVF = intravenous fluids; TPN = total parenteral nutrition

6.3.7 Management of Cardiac Events

6.3.7.1 Management of Cardiac Instability

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

6.3.7.2 Management of Left Ventricular Dysfunction

Guidance for TAK-228 dose adjustment for the event of left ventricular dysfunction is provided in the table below.

Table 11: Management of Left Ventricular Dysfunction

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

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

6.3.7.3 Management of QTc Prolongation

Guidance for TAK-228 dose adjustment for the event of QTcF prolongation is provided in the table below.

Table 12: Management of QTc Prolongation

Grade	Description	Treatment	TAK-228 Dose Modification
2	480 ms < QTcF < 501 ms	Evaluate for other possible causes (eg, electrolyte disturbance, concomitant medication, etc.)	None; continue TAK-228 at the same dose and schedule.
≥ 3	QTcF ≥ 501 ms	Evaluate for other possible causes (eg, electrolyte disturbance, concomitant medication)[a]; Consider a formal consult by a cardiologist; Notify the study doctor; Additional ECGs may be performed at intervals that the treating physician deems clinically appropriate until repeated QTc measurements fall or are below the threshold interval that triggered the repeat measurement.	TAK-228 should be interrupted. Subjects who experience persistent symptomatic Grade 3 or Grade 4 QTc prolongation without another cause should permanently discontinue study treatment.

Abbreviations: ECG = electrocardiogram; IV = intravenous; ms = milliseconds; QTc = QT interval corrected for heart rate

a A list of medications known to prolong QTc can be found at www.torsades.org and www.QTdrugs.org.

6.3.8 Management of Aspartate Aminotransferase/Alanine Aminotransferase Elevations

Table 14: Management of Aspartate Aminotransferase/Alanine Aminotransferase 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 subject for other causes of transaminitis (eg, 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 (ie, Hy's Law);
4	>20×ULN	Same as for Grade 2.	Stop TAK-228 and discontinue subject from the study.

Table 14: Management of Aspartate Aminotransferase/Alanine Aminotransferase Elevations

Grade	Description	Treatment	Dose Modification
			Permanently discontinue study treatment if in combination with Grade 2 total bilirubin elevation when alternative causes cannot be identified (ie, Hy's Law).
Prevention/Prophylaxis:			
Ensure proper screening of subjects for study participation.			

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

Patients meeting Hy's Law criteria regardless of attribution or of alternative etiology, must discontinue treatment.

6.3.9 Management of Other Non-Hematologic Toxicities

Guidance for TAK-228 dose management in the event of non-hematologic toxicities is provided in the table below.

Table 13: 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, no adjustment required. • If toxicity becomes intolerable, hold TAK-228 until recovery to ≤Grade 1, then reinitiate at same dose.
≥ 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated		Hold TAK-228 until recovery to ≤ Grade 1. Reinitiate TAK-228 at dose reduced by 1 level. Subjects 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 as determined by PI, in which case they may be retreated at a reduced dose level after recovery to ≤ Grade 1 severity.

7. STUDY CALENDAR & EVALUATIONS

Study Evaluation Cycle = 28 days	Screening prior to registration	On Treatment Cycle 1 ± 3 days				On Treatment Cycle 2 ± 3 days		On Treatment Cycle 3 ± 3 days		Subsequent Cycles ± 3 days	Every 8 weeks ± 7 days	Treatment Discontinuation Visit[13] (± 7 days)	Follow up[14]
	-28 days	Day 1	Day 8	Day 15	Day 22	Day 1	Day 15	Day 1	Day 15	Day 1		30 days of last treatment	
REQUIRED ASSESSMENTS													
Informed Consent	X												
Medical History	X												
Physical Exam	X	X	X	X	X	X	X	X	X	X		X	
Vital signs and ECOG Performance Status[1]	X	X	X	X	X	X	X	X	X	X		X	
ECG	X	X				X		X		X		X	
Cardiac echo or MUGA	X												
AEs & concomitant medications	X	X	X	X	X	X	X	X	X	X		X	
LABORATORY ASSESSMENTS													
Complete Blood Cell Count with diff (CBC)[2]	X	X	X	X	X	X	X	X	X	X		X	
Comprehensive Metabolic Profile (CMP)[3]	X	X	X	X	X	X	X	X	X	X		X	
LDH	X												
Fasting Lipid Profile[4]	X	X				X		X		X	X	X	
PT/INR and aPTT	X	X				X		X		X			
TSH	X	X		X		X	X					X	
HbA1c[5]	X							X		X			
In home daily fasting glucose monitoring[6]			X	X	X	X	X	X	X	X	X		
Pregnancy test (serum or urine) (WOCBP)	X												
Urinalysis[7]	X	X	X	X	X	X	X	X	X	X		X	
DISEASE ASSESSMENT													
CT of chest	X										X		

Study Evaluation Cycle = 28 days	Screening prior to registration	On Treatment Cycle 1 ± 3 days				On Treatment Cycle 2 ± 3 days		On Treatment Cycle 3 ± 3 days		Subsequent Cycles ± 3 days	Every 8 weeks ± 7 days	Treatment Discontinuation Visit[13] (± 7 days)	Follow up[14]
	-28 days	Day 1	Day 8	Day 15	Day 22	Day 1	Day 15	Day 1	Day 15	Day 1		30 days of last treatment	
CT or MRI of abdomen and pelvis[8]	X										X		
MRI or CT Brain[9]	X												
TREATMENT EXPOSURE													
TAK-228 (D1, D8, D15, D22)		X	X	X	X	X	X	X	X	X			
SPECIMEN COLLECTION													
Archival Tumor Tissue[10]	X												
Tumor Biopsy[11]	X											X	
Blood sample for Germline DNA analysis[12]		X											
Blood Sample for Biomarker analysis[13]		pre-dose									X	X	
FOLLOW-UP													
Survival Status and Subsequent Therapy													X

Key to Footnotes

1: Vital signs to include blood pressure, oxygen saturation (screening only) heart rate, respiratory rate, body temperature, weight, height (screening only) and ECOG performance status

2: Hematology testing to include full CBC with WBC, ANC, hemoglobin, and platelet count and differential.

3: Comprehensive metabolic panel to include sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, albumin, AST, ALT, total bilirubin, magnesium. Patients should be fasting for at least 8 hours prior to lab collection for day 1 only of each cycle.

4: Fasting is defined as nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment.

5: HbA1c is required at screening, C3D1 and every 3 subsequent cycles.

6: Fasting serum glucose will be measured in the clinic at each clinic visit. Subjects are required to fast overnight (nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment) for each of these measurements. In-home glucose monitoring will begin on Cycle 1 Day 2 and is required daily for the first two months of treatment. If levels remain stable, monitoring can be decreased to once a week after the first two cycles, otherwise it is required daily until the last dose of study drug. In-home glucose monitoring is not required on the days that subjects will be getting fasting serum glucose testing in clinic. Please see the Management of Hyperglycemia section for details.

7: Subjects with ≥ 2+ protein on dipstick urinalysis must undergo 24-hour urine collection for protein

8: Diagnostic CT chest and CT or MRI of the abdomen and pelvis should be obtained at screening and at every 8 week imaging assessment relative to the C1D1 visit. If doses are held due to toxicity, scans should continue on this every 8 week interval relative to C1D1.

9: MRI of the brain with and without contrast is preferred. If a subject is not able to obtain an MRI, CT imaging with contrast is acceptable. If a subject is not able to receive contrast, CT head without contrast is acceptable.

10: Archival tissue needs to be requested and received prior to therapy initiation if available.

11: Baseline fresh tumor biopsies are MANDATORY for subjects that do not have archival tissue available from the previous 18 months. Screening tumor biopsies must be collected within 28 days prior to initiation of study therapy. Progression tumor biopsies are MANDATORY ONLY in subjects who experience an initial disease response per RECIST 1.1 criteria and then subsequent progression, if feasible and safe.

12: MANDATORY: Blood for germline DNA analysis will be collected prior to C1D1 treatment. MANDATORY: Research blood samples for biomarker analysis to be collected prior to C1D1 treatment and every 8 weeks until off treatment. If off treatment collection is within 2 weeks of prior collection, research samples do not need to be collected.

13: Subjects who discontinue study treatment will return for a treatment discontinuation visit around 30 days (± 7 days) after the last study treatment. The visit at which a response assessment shows progressive disease may be used as the treatment discontinuation visit.

14: After a subject discontinues from study treatment, they will continue to be followed for survival and subsequent anti-cancer therapy every 6 months until death or 2 years after study treatment discontinuation. Follow up may be accomplished via clinic visit, phone call, or other avenues as appropriate.

7.1 Screening Evaluations

Within 28 days prior to registration for protocol therapy

- Informed consent
- Medical History
- Diagnosis and staging assessment
- Physical Examination including review of medications (over-the-counter or prescribed) and side effects the subject is experiencing
- Vital signs (including temperature, heart rate, blood pressure, weight, height and oxygen saturation) and ECOG performance status
- ECG
- Cardiac Echo or MUGA
- Laboratory Testing
    - CBC with differential and platelet count
    - Comprehensive Metabolic Profile
    - PT/INR and aPTT
    - Thyroid Function Tests (TSH)
    - Pregnancy Test (urine or serum) for WOCBP
    - Fasting Lipid Profile (fasting is defined as nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment).
    - HbA1c
    - LDH
    - Urinalysis (Subjects with ≥ 2+ protein on dipstick urinalysis at baseline must undergo 24-hour urine collection for protein)
- Disease assessments (imaging with contrast is preferred unless contraindicated)
    - CT of Chest
    - CT or MRI of Abdomen and Pelvis
    - MRI Brain with/without contrast preferred; other options include: CT imaging with contrast or CT without contrast if the subject cannot receive contrast. CT head without contrast is acceptable
- Research Testing to be done after confirmation of eligibility
    - Archival tissue will be requested and received prior to C1D1. Formalin-fixed, paraffin-embedded (FFPE) tumor tissue block is preferred. Alternatively, a minimum of 20unstained, charged, paraffin coated slides will suffice. Fine-needle aspiration, brushings, cell pellet from pleural effusion, bone marrow aspirate/biopsy are not acceptable.
    - MANDATORY: Pre-treatment biopsy should take place prior to initiation of protocol therapy C1D1 following confirmation of eligibility if the subject does not have archival tissue from the previous 18 months available.

7.2 On Treatment Evaluations (± 3 days)

Cycle 1 Day 1

- Physical Examination including review of medications (over-the-counter or prescribed) and side effects the subject is experiencing
- Vital signs (including temperature, heart rate, blood pressure, weight, height and oxygen saturation) and ECOG performance status
- ECG
- In home glucose monitoring:
    - Fasting serum glucose will be measured in the clinic at each clinic visit. Subjects are required to fast overnight (nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment) for each of these measurements. In-home glucose monitoring will begin on Cycle 1 Day 2 and is required daily for the first two months of treatment. If levels remain stable, monitoring can be decreased to once a week after the first two cycles, otherwise it is required daily until the last dose of study drug. In-home glucose monitoring is not required on the days that subjects will be getting fasting serum glucose testing in clinic.
- Laboratory Testing
    - CBC with differential and platelet count
    - Comprehensive Metabolic Profile
    - PT/INR and aPTT
    - Thyroid Function Tests (TSH)
    - Fasting Lipid Profile (fasting is defined as nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment).
    - Urinalysis (Subjects with ≥ 2+ protein on dipstick urinalysis at baseline must undergo 24-hour urine collection for protein)
- MANDATORY: Research Blood Samples prior to initiation of protocol therapy C1D1
    - Germline DNA analysis
    - Blood biomarker analysis
- Medication Administration: TAK228 (D1, D8, D15, D22)

Cycle 1 Day 8

- Physical Examination including review of medications (over-the-counter or prescribed) and side effects the subject is experiencing
- Vital signs (including temperature, heart rate, blood pressure, weight, height and oxygen saturation) and ECOG performance status
- In home glucose monitoring:
    - Fasting serum glucose will be measured in the clinic at each clinic visit. Subjects are required to fast overnight (nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment) for each of these measurements. In-home glucose monitoring will be required daily for the first two months of treatment. In-home glucose monitoring is not required on the days that subjects will be getting fasting serum glucose testing in clinic.
- Laboratory Testing
    - CBC with differential and platelet count
    - Comprehensive Metabolic Profile
    - Urinalysis (Subjects with ≥ 2+ protein on dipstick urinalysis at baseline must undergo 24-hour urine collection for protein)

- Medication Administration: TAK228 (D1, D8, D15, D22)

Cycle 1 Day 15

- Physical Examination including review of medications (over-the-counter or prescribed) and side effects the subject is experiencing
- Vital signs (including temperature, heart rate, blood pressure, weight, height and oxygen saturation) and ECOG performance status
- In home glucose monitoring:
    - Fasting serum glucose will be measured in the clinic at each clinic visit. Subjects are required to fast overnight nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment) for each of these measurements. In-home glucose monitoring will be required daily for the first two months of treatment. In-home glucose monitoring is not required on the days that subjects will be getting fasting serum glucose testing in clinic.
- Laboratory Testing
    - CBC with differential and platelet count
    - Comprehensive Metabolic Profile
    - TSH
    - Urinalysis (Subjects with ≥ 2+ protein on dipstick urinalysis at baseline must undergo 24-hour urine collection for protein)
- Medication Administration: TAK228 (D1, D8, D15, D22)

Cycle 1 Day 22

- Physical Examination including review of medications (over-the-counter or prescribed) and side effects the subject is experiencing
- Vital signs (including temperature, heart rate, blood pressure, weight, height and oxygen saturation) and ECOG performance status
- In home glucose monitoring:
    - Fasting serum glucose will be measured in the clinic at each clinic visit. Subjects are required to fast overnight (nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment) for each of these measurements. In-home glucose monitoring is not required on the days that subjects will be getting fasting serum glucose testing in clinic.
- Laboratory Testing
    - CBC with differential and platelet count
    - Comprehensive Metabolic Profile
    - Urinalysis (Subjects with ≥ 2+ protein on dipstick urinalysis at baseline must undergo 24-hour urine collection for protein)
- Medication Administration: TAK228 (D1, D8, D15, D22)

Cycle 2 Day 1

- Physical Examination including review of medications (over-the-counter or prescribed) and side effects the subject is experiencing
- Vital signs (including temperature, heart rate, blood pressure, weight, height and oxygen saturation) and ECOG performance status
- In home glucose monitoring:

  - Fasting serum glucose will be measured in the clinic at each clinic visit. Subjects are required to fast overnight (nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment) for each of these measurements. In-home glucose monitoring is not required on the days that subjects will be getting fasting serum glucose testing in clinic.
- ECG
- Laboratory Testing
  - CBC with differential and platelet count
  - Comprehensive Metabolic Profile
  - PT/INR and aPTT
  - Thyroid Function Tests (TSH)
  - Fasting Lipid Profile (fasting is defined as nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment).
  - Urinalysis (Subjects with ≥ 2+ protein on dipstick urinalysis at baseline must undergo 24-hour urine collection for protein)
- Medication Administration: TAK228 (D1, D8, D15, D22)

Cycle 2 Day 15

- Physical Examination including review of medications (over-the-counter or prescribed) and side effects the subject is experiencing
- Vital signs (including temperature, heart rate, blood pressure, weight, height and oxygen saturation) and ECOG performance status
- In home glucose monitoring:
  - Fasting serum glucose will be measured in the clinic at each clinic visit. Subjects are required to fast overnight (nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment) for each of these measurements. In-home glucose monitoring is not required on the days that subjects will be getting fasting serum glucose testing in clinic.
- Laboratory Testing
  - CBC with differential and platelet count
  - Comprehensive Metabolic Profile
  - TSH
  - Urinalysis (Subjects with ≥ 2+ protein on dipstick urinalysis at baseline must undergo 24-hour urine collection for protein)
- Medication Administration: TAK228 (D1, D8, D15, D22)

Cycle 3 Day 1

- Physical Examination including review of medications (over-the-counter or prescribed) and side effects the subject is experiencing
- Vital signs (including temperature, heart rate, blood pressure, weight, height and oxygen saturation) and ECOG performance status
- In home glucose monitoring:
  - Fasting serum glucose will be measured in the clinic at each clinic visit. Subjects are required to fast overnight (nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment) for each of these

measurements In-home glucose monitoring is not required on the days that subjects will be getting fasting serum glucose testing in clinic.

- ECG
- Laboratory Testing
    - CBC with differential and platelet count
    - Comprehensive Metabolic Profile
    - PT/INR and aPTT
    - HbA1c
    - Fasting Lipid Profile (fasting is defined as nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment).
    - Urinalysis (Subjects with ≥ 2+ protein on dipstick urinalysis at baseline must undergo 24-hour urine collection for protein)
- Medication Administration: TAK228 (D1, D8, D15, D22)

Cycle 3 Day 15

- Physical Examination including review of medications (over-the-counter or prescribed) and side effects the subject is experiencing
- Vital signs (including temperature, heart rate, blood pressure, weight, height and oxygen saturation) and ECOG performance status
- In home glucose monitoring:
    - Fasting serum glucose will be measured in the clinic at each clinic visit. Subjects are required to fast overnight (nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment for each of these measurements. If levels remain stable after 2 months of treatment, monitoring can be decreased to once a week after the first two cycles, otherwise it is required daily until the last dose of study drug. In-home glucose monitoring is not required on the days that subjects will be getting fasting serum glucose testing in clinic.
- Laboratory Testing
    - CBC with differential and platelet count
    - Comprehensive Metabolic Profile
    - Urinalysis (Subjects with ≥ 2+ protein on dipstick urinalysis at baseline must undergo 24-hour urine collection for protein)
- Medication Administration: TAK228 (D1, D8, D15, D22)

Cycle 4 Day 1 and subsequent Cycles

- Physical Examination including review of medications (over-the-counter or prescribed) and side effects the subject is experiencing
- Vital signs (including temperature, heart rate, blood pressure, weight, height and oxygen saturation) and ECOG performance status
- In home glucose monitoring:
    - Fasting serum glucose will be measured in the clinic at each clinic visit. Subjects are required to fast overnight (nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment) for each of these measurements. If levels remain stable after 2 months of treatment, monitoring can be decreased to once a week after the first two cycles, otherwise it is required

daily until the last dose of study drug. In-home glucose monitoring is not required on the days that subjects will be getting fasting serum glucose testing in clinic.

- ECG
- Laboratory Testing
    - CBC with differential and platelet count
    - Comprehensive Metabolic Profile
    - PT/INR and aPTT
    - HbA1c
    - Urinalysis (Subjects with ≥ 2+ protein on dipstick urinalysis at baseline must undergo 24-hour urine collection for protein)
    - Fasting Lipid Profile (fasting is defined as nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment).
- Medication Administration: TAK228 (D1, D8, D15, D22)

Every 8 weeks (± 7 days)

- Laboratory
    - Fasting Lipid Profile
    - Blood for biomarker analysis
- In home glucose monitoring:
    - Fasting serum glucose will be measured in the clinic at each clinic visit. Subjects are required to fast overnight (nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment) for each of these measurements. If levels remain stable after 2 months of treatment, monitoring can be decreased to once a week after the first two cycles, otherwise it is required daily until the last dose of study drug. In-home glucose monitoring is not required on the days that subjects will be getting fasting serum glucose testing in clinic.
- Disease assessments- scans are done every 8 weeks relative to the C1D1 visit (imaging with contrast is preferred unless contraindicated)
    - CT of Chest
    - CT or MRI of Abdomen and Pelvis

7.3 Safety Follow-up Visit Evaluations

Subjects discontinued from the treatment phase of the study for any reason will be evaluated 30 days (± 7) after the last dose of study drug. Discuss the sequence of events that should occur during the visit, e.g., review of medications, assessment of adverse events, etc. These should be consistent with the Study Calendar.

- Physical Examination including review of medications (over-the-counter or prescribed) and side effects the subject is experiencing
- Vital signs (including temperature, heart rate, blood pressure, weight, height and oxygen saturation) and ECOG performance status
- ECG
- Laboratory Testing
    - CBC with differential and platelet count
    - Comprehensive Metabolic Profile
    - Thyroid Function Tests (TSH)

  - Fasting Lipid Profile (fasting is defined as nothing except water and/or medications after midnight or for a minimum of 8 hours before the assessment).
  - Urinalysis (Subjects with ≥ 2+ protein on dipstick urinalysis at baseline must undergo 24-hour urine collection for protein)
- Progression tumor biopsies are MANDATORY ONLY in subjects who experience an initial disease response per RECIST 1.1 criteria and then subsequent progression, if feasible and safe.

7.4 Long Term Follow-up Evaluations

After a subject discontinues from study treatment, they will continue to be followed for survival and subsequent anti-cancer therapy every 6 months until death or 2 years after study treatment discontinuation. Follow up may be accomplished via clinic visit, phone call, or other avenues as appropriate.

8. CORRELATIVE TISSUE AND BLOOD ANALYSES

8.1 TISSUE ANALYSES

A DFCI mutation screen analysis of 300 cancer genes (Oncopanel), including all those involved in regulation of mTOR will be performed on pre-treatment biopsies in all subjects to correlate with response to TAK-228(22). Data will be analyzed using standard pipeline tools for the CLIA use of this mutation analysis to identify sequence variants present in the tumor and not in the normal DNA sample, as well as tools to identify regions of significant copy number variation across the genome.

Immunoblotting and IHC will performed on pre and post-treatment biopsies to analyze the downstream targets of mTORC1 and mTORC2 and correlate with the clinical response to TAK-228. The downstream targets are the following: S6, p-S6, S6K1, p-S6K1, 4EBP1, p-4EBP1, Akt-S473 and p-AKT-S473.

In responding subjects who subsequently progress, the post-treatment biopsy will be used for genetic studies to determine the mechanism of resistance. A DFCI mutation screen (Oncopanel) as well as whole exome sequencing will be performed on pre and post-treatment biopsies to understand the mechanism of resistance.

8.1.1 Archival Tissue Correlative Studies

Archival tissue, preferred to have been collected within 18 months of enrollment otherwise, will be obtained prior to study treatment if available. This tissue will be used to assess response to TAK-228, and evaluate mechanisms of acquired resistance to therapy. Mutation profiling will be performed using massively parallel sequencing technology (Oncopanel) and/or whole exome sequencing. In addition, nucleic acid purification will be performed on the specimens.

The archival specimen must contain adequate viable tumor tissue and be overall representative of the whole tumor (i.e. containing predominant and highest Fuhrman grade areas). Formalin-fixed paraffin-embedded tumor tissue blocks are preferred and should contain tumor areas that measure at least 1 cm square in aggregate. Alternatively, a minimum of 20 unstained 4-micron-thick sections (on charged slides) can be provided. The unstained slides should be coated with paraffin. Surgical specimens are preferred with order of preference being 1) nephrectomy specimens and 2) metastatectomy specimens. If surgical specimens are not available, core-needle biopsy specimens are acceptable. Fine-needle aspiration, brushings, cell pellet from pleural effusion, bone marrow aspirate/biopsy are not acceptable.

8.1.2 Fresh Tissue Correlative Studies

8.1.2.1 Pre-Treatment

If subjects do not have archival tissue from within the previous 18 months available, they will undergo one mandatory biopsy prior to study treatment. The pretreatment biopsy must occur within 28-days prior to initiation of study therapy.

8.1.2.2 Progression

In subjects who experience an objective response as defined by RECIST version 1.1 and then subsequent progression as defined by RECIST 1.1, a biopsy will be mandated at the time of disease progression from a site of metastasis, if feasible and safe. Subjects should continue study treatment until the day prior to the progression biopsy. Progression biopsy must occur within 28-days of determination of disease progression.

The metastatic sites need to be safely accessible as deemed by the site investigator and interventional radiologist. During the core needle biopsy procedure, four core samples will be collected: one will be placed in formalin for routine histology and immunohistochemistry (IHC) studies and the remaining three will be frozen in liquid nitrogen. Two of the cores will be utilized for genomic analysis and the remaining core will be used for IHC and immunoblot analysis. In addition, nucleic acid purification will be performed on the specimens.

Biopsies will be obtained in a manner that minimizes risk and will be taken only if there is no intervening condition (e.g., thrombocytopenia or neutropenia) that, in the opinion of the site investigator, increases the likelihood of procedural complications to an unacceptable level.

Biopsies should be performed per institutional standards and/or operator preference. Blood samples will be drawn within 2 weeks of the biopsy to document an acceptable coagulation profile (INR $\leq$ 1.5, PTT $\leq$ 60, platelets > 50,000). Aspirin, clopidogrel, and NSAIDS should be discontinued 5 days prior to the biopsy.

Preferred biopsy sites include: lymph nodes, peripheral based liver lesions, exophytic soft tissue components associated with bone lesions, subcutaneous nodules, pleural-based lesions, and kidney lesions. An 18 gauge needle or larger is preferred when possible. For each tissue

collection procedure, the intent is to acquire at a minimum of 4 cores and up to 6 cores. Though the size of the biopsy cores is variable, to optimize tumor capture, larger cores are preferred.

8.2 BLOOD ANALYSES

8.2.1 Blood for Germline DNA

- This sample will be collected once prior to C1D1.
- Five (5) mL of blood will be collected

8.2.2 Blood for cfDNA Biomarker Research

- This sample will be collected prior to C1D1 treatment and every 8 weeks until off treatment. If off treatment collection is within 2 weeks of prior collection, research samples do not need to be collected.
- 10 mL of blood will be collected

8.3 Confidentiality of Biospecimens

Samples that are collected will be identified by a subject's study number assigned at the time of registration to the trial. Any material issued to collaborating researchers will be anonymized and only identified by the subject's study number.

9. CRITERIA FOR DISEASE EVALUATION

For the purposes of this study, subjects should be re-evaluated for response every 8 weeks. Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) (23). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

9.1 Definitions

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

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

9.2 Disease Parameters

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

NOTE: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

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

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

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

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

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

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

9.3 Methods for Evaluation of Disease

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

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

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.
Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

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

9.4 Response Criteria

9.4.1 Evaluation of Target Lesions

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

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an

absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

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

9.4.2 Evaluation of Non-Target Lesions

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

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

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

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

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

9.4.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

9.4.4 Evaluation of Best Overall Response

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

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

Table 17: Definition of clinical response for subjects with measurable disease

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

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

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

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

Table 18: Definition of clinical response for subjects with non-measurable disease

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

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

9.4.5 Duration of Response

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

disease the smallest measurements recorded since the treatment started, or death due to any cause. Subjects without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Subjects without events reported are censored at the last disease evaluation.

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

9.4.6 Response Review

Tumors will be assessed for response and progression by RECIST version 1.1 by central radiology review.

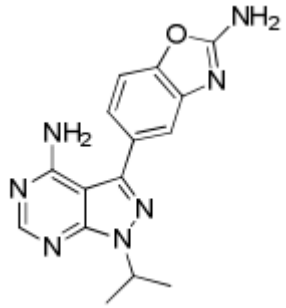
10. DRUG INFORMATION

Please refer to the current version of the Investigator's Brochure (IB) for additional information regarding this drug.

10.1 TAK-228

The chemical name and structure for the active form of TAK-228 are provided in the following:

- Research Name: TAK-228
  INK128
  I-119
- Chemical Names: 3-(2-amino-1,3-benzoxazol-5-yl)-1-(propan-2-yl)-1H-pyrazolo [3, 4-d] pyrimidin-4-amine
- Molecular Formula: C 15 H 15 N 7 O
- CAS Registry Number: 1224844-38-5
- CAS Registry Name: 1H-Pyrazolo[3,4-d]pyrimidin-4-amine,3-(2-amino-5-benzoxazolyl)-1-(1-methylethyl)-
- Molecular Weight: 309.3 g/mol
- Chemical Structure:



The milled API, may result in faster absorption profile with possibly higher maximum concentration (Cmax), which could result in a different safety profile compared to the previous unmilled API capsules. Therefore, ongoing studies (C31001, C31002 and , TAK-228-1004 –A Phase I, open label study to evaluate the safety, tolerability, and pharmacokinetics of TAK-228 as a single agent and in combination with paclitaxel in adult subjects with advanced non-hematologuical malignancies-), with the new milled API will determine the recommended phase 2 dose (RP2D) for single agent 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.

Overall, PK data from Studies INK128-001, INK128-002, and INK128-003 indicate that TAK-228 exhibits fast oral absorption (time to reach the maximal concentration (Cmax) [tmax],generally between 1-4 hours after dosing); has dose-linear PK, with a mean plasma half-life of approximately 8 hours; and does not accumulate meaningfully in plasma when dosed as frequently as once daily and under any of 4 tested dosing regimens (1.once daily, 2. once daily for 3 consecutive days followed by a 4-day dosing holiday every week, 3.once daily for 5 consecutive days followed by a 2-day dosing holiday every week, 4.once weekly). The PK of TAK-228 was generally consistent, with no appreciable differences across the clinical studies that measured PK. Neither paclitaxel nor TAK-228 appeared to alter the PK of the other agent when co-administered.
Clinical Drug-Drug Interaction (DDIs) 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 drug-drug interaction. 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.

10.2 Supplier/How Supplied

Millennium will supply TAK-228 at no charge to subjects participating in this clinical trial.

TAK-228 drug substance is a white to off-white crystalline powder. The values of acid dissociation constant in logarithmic scale (pKa) were determined to be 2.9 and 4.1.
TAK-228 drug product has been developed as an immediate-release capsule for oral administration. The drug product contains a mixture of drug substance, microcrystalline cellulose, and magnesium stearate encapsulated in size 2 hard-gelatin capsules. Clinical trial materials are supplied as capsules in 1.0- (white, opaque), 3.0- (swedish orange, opaque), and 5.0 mg (gray, opaque) dosage strengths.

10.3 Preparation

TAK-228 study drug will be provided in 60 cc high-density polyethylene (HDPE) bottles with polypropylene, child-resistant caps and induction seal. Study drug will be dispensed with dosing instructions for home use, including the requirement that capsules are stored in their original containers and that capsules be swallowed whole and not opened, chewed, or manipulated in any way. Materials provided by the sponsor should be dispensed to subjects with clear administration instructions from the site investigator.

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

10.4 Storage and Stability

TAK-228 capsules are packaged either in blister packs or in induction-sealed, high-density polyethylene bottles equipped with child-resistant caps. TAK-228 drug product is stable when stored in its original packaging between 15°C to30°C, with allowed short-term excursions between 2°C to 40°C.

All temperature excursions will be reported for assessment and authorization for continued use. All investigational supplies must be stored in a secure area with controlled access and will be stored in original packaging. All drug supplies should be used before the retest expiry date.

10.5 Handling and Disposal

TAK-228 drug product is an anticancer drug. As with other anticancer compounds, caution should be exercised when handling TAK-228. It is recommended that gloves and protective garments be worn during preparation when dispensed in the clinic. Please refer to published guidelines regarding the proper handling and disposal of anticancer agents. 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 clean-up operation. The product may be harmful if inhaled, ingested, or absorbed through the skin. Gloves and protective clothing should be worn during the clean-up operation. The area should be ventilated and the spill site washed after material pick-up is complete. The spilled material should be disposed of as hazardous medical waste in compliance with federal, state, and local regulations. In case of contact with the powder (eg, from a broken capsule), 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.

Drug should be destroyed at the site, after the site investigator approves the drug destruction policy at the site. Drug will not be returned to Millennium. Destruction will be documented in the Drug Accountability Record Form.

10.6 Accountability

Accountability for investigational product is the responsibility of the site investigator. The study site must maintain accurate records demonstrating dates and amount of investigational product received, to whom dispensed (subject by subject accounting), and accounts of any investigational product accidentally or deliberately destroyed. At the end of the study, reconciliation must be made between the amount of investigational product supplied, dispensed, and subsequently destroyed. At the time of delivery of investigational product to the site, the site investigator, designee, or pharmacist (where appropriate) will confirm that the supplies for the study have

been received. This following information will be confirmed: lot numbers, quantities shipped/delivered, and date of receipt.

11. ADVERSE EVENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 11.1) and the characteristics of an observed AE (Section 11.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

11.1 Expected Toxicities

11.1.1 Adverse Event List

11.1.1.1 Adverse Event List for TAK-228

Among the 335 subjects who had received ≥1 dose of study drug as of the clinical data cutoff date (09 December 2014), 18 deaths had occurred within 30 days of the last dose, including 7 in Study INK128-001, 2 in Study INK128-002, and 9 in Study INK128-003. One death (ventricular fibrillation and cardiac arrest; Study INK128-001) was considered related to TAK-228. At least 1 treatment-emergent SAE, regardless of causality, had been reported in 125/335 subjects (37%). Across the studies and regardless of causality or dosing regimen, the most common treatment emergent adverse events (TEAEs) included nausea, fatigue, hyperglycemia, vomiting, diarrhea, stomatitis, and decreased appetite.

11.2 Adverse Event Characteristics

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

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

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

11.3 DF/HCC Expedited Adverse Event Reporting

11.3.1 Investigators must report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

11.3.2 For multi-institution studies where a DF/HCC investigator is serving as the Overall Principal Investigator, each participating institution must abide by the reporting requirements set by the DF/HCC. This applies to any medical event equivalent to an unexpected grade 2 or 3 with a possible, probable or definite attribution, grade 4 toxicities, and grade 5 (death) regardless of study phase or attribution.

11.3.3 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

Other investigative sites will report AEs to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional AE form should be forwarded to the Overall PI within the timeframes detailed in the table below.

	DF/HCC Reportable AEs				
Attribution	**Gr. 2 & 3 AE Expected**	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days[#]	5 calendar days	24 hours*
Possible Probable Definite	Not required	5 calendar days	5 calendar days[#]	5 calendar days	24 hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, the AE should be reported within 1 business day of learning of the event.					

The Overall PI will submit AE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting adverse events

11.4 Expedited Reporting to Millennium

11.4.1 Definitions

11.4.1.1 Serious Adverse Event (SAE)

A SAE is an adverse event that:

- Results in death. NOTE: Death due to disease progression should not be reported as a SAE, unless it is attributable by the site investigator to the study drug(s)
- Is life-threatening (defined as an event in which the subject 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 for >24 hours or prolongation of existing hospitalization. **NOTE:** Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions not resulting in hospitalization; or the development of drug dependency or drug abuse.

11.4.1.2 Unexpected Adverse Event

For this study, an AE is considered unexpected when it varies in nature, intensity or frequency from information provided in the current IB, prescribing information or when it is not included in the informed consent document as a potential risk. Unexpected also refers to AEs that are mentioned in the IB as occurring with a class of drugs or are anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

11.4.2 Requirements for Reporting SAEs to Millennium

Adverse Events 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.

Adverse Events which are **serious** 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). In addition, new primary malignancies that occur during the follow-up periods must be reported, regardless of causality to study regimen, for a minimum of two years after the last dose of the investigational product, starting from the first dose of study drug. All new cases of primary malignancy 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 principal investigator Dr. Bradley McGregor also referred to as the sponsor-investigator, is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the sponsor- investigator's EC or IRB.

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.

See below for contact information for the reporting of SAEs to Millennium Pharmacovigilance.

The sponsor-investigator must fax or email the SAE Form per the timelines above. A sample of an SAE Form will be provided.

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 by 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 Takeda 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 his/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)?

US and Canada
Toll-Free Fax #: [redacted]

All other countries (Rest of World)
[redacted]

11.4.3 Procedures for Reporting Drug Exposure During Pregnancy and Birth Events

If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately and permanently discontinue study drug. The sponsor-investigator must fax a completed Pregnancy Form to the Takeda Pharmacovigilance or designee immediately (see Section 11.4.2). 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 (see Section 11.4.2). Every effort should be made to follow the pregnancy for the final pregnancy outcome.

11.4.4 Requirements for Reporting Product Complaints to Millennium

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact Millennium Pharmaceuticals (see below) and report the event. Whenever possible, the associated product

should be maintained in accordance with the label instructions pending further guidance from a Millennium Pharmaceuticals Quality representative.

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

For Product Complaints or Medication Errors, call

For ADCETRIS or PIPELINE Products:
Phone: [redacted]
Email: [redacted]

Product complaints in and of themselves are not AEs. If a product complaint results in an SAE, a MedWatch 3500a should be completed and sent to Millennium Pharmaceuticals Pharmacovigilance

11.5 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

11.6 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

11.7 Routine Adverse Event Reporting

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

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

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) should not be recorded as an AE.

Disease progression should not be recorded as an AE, unless it is attributable to the study regimen by the site investigator.

12. STATISTICAL METHODS

12.1 Study Design

This is an open-label, single-arm phase II study of TAK-228 (the ATP-competitive inhibitor of the serine/threonine kinase mTOR) at recommended phase II dosing of 30 mg orally once per week, in subjects with previously treated advanced RCC. The study consists of a two-stage design. The first stage will assess futility when half of the planned patients are enrolled and have had their first (8 week) imaging assessment to determine their response status. The second stage will assess the overall response after all patients are enrolled.

12.2 Endpoints

12.2.1 Definition of Primary Endpoint

Objective Response (OR): Confirmed CR or PR as best overall response according to RECIST version 1.1 by central review. Objective response will be determined by the best overall confirmed response designation recorded between the date of first dose of trial therapy and the date of objectively documented disease progression or cessation of trial therapy, whichever occurs first. For subjects without documented progression or cessation of trial therapy, all available response designations will contribute to the objective response determination.

12.2.2 Definition of Secondary Endpoints

- Safety and tolerability according to NCI CTCAE version 4.0
- Progression-free survival (PFS): time from registration to the earlier of (radiographic) progression based on RECIST 1.1 or death due to any cause. Subjects alive without disease progression are censored at date of last disease evaluation.
- Overall survival (OS): time from registration to death due to any cause, or censored at date last known alive.

12.3 Sample Size and Accrual

Forty subjects with metastatic RCC will be enrolled for a goal of 38 evaluable subjects (assuming 5% not evaluable). ,The two-stage single arm phase II study is designed to detect an improvement in response rate from 5% to 20%. Assuming a type I error of 0.05 and targeting 90% statistical power, 20 patients will be accrued (for 19 evaluable) in the first stage. The first interim analysis will occur when the first 8 week imaging assessment for response data is available for all 20

patients. If 1 or more responses are observed, the accrual will continue to the second stage. Twenty (for 19 evaluable) additional patients will be accrued in the second stage. TAK-228 will be declared ineffective in this study if <5 responses are observed. It will be considered worthy of further study if more than 4 (≥5) responses are observed.

The design operating characteristics are as follows:

Hypothesis	#s responses at stage I	Prob(stop at first stage	Overall # responses	Prob(Rx declared inactive)	Design Statistics
Ho: ORR=0.05	<1	38%	≤4	96%	True type I error 0.04
Ha: ORR=0.2	<1	1.4%	≤4	10%	Power: 90%

The 90% confidence intervals for the response rate in a two-stage phase II design are summarized as below. The calculation employed the method of Atkinson and Brown (1985) Biometrics 741-744

Observed # of responses	Response rate	90% CI
4/38	0.105	(0.04, 0.23)
5/38	0.13	(0.05, 0.26)
6/38	0.16	(0.07, 0.29)
7/38	0.18	(0.09, 0.32)

The accrual is expected to be 3 subjects every month for 14 months to complete enrollment with additional follow-up to 2 years for disease progression or survival. The study is expected to last approximately 40 months.

12.4 Analysis Datasets

Population	Definition
Enrolled	This will comprise all subjects who meet the eligibility criteria and are registered onto the study.
Evaluable	Subjects who receive at least one dose of trial drug TAK-228 and have at least one response assessment or die before any evaluation.
Safety	All subjects receiving at least one dose of TAK-228 will be included in analysis unless otherwise specified.

12.4.1 Analysis Plans for Primary Objective

ORR is defined as the proportion of subjects who achieve objective response, as defined in Section 12.2.1. ORR and its 90% exact confidence interval (CI) using the method of Atkinson and Brown (1985) will be provided based on the evaluable population. Best overall response (BOR) will also be summarized. In addition, tumor burden data will be summarized by waterfall plot showing the maximum tumor shrinkage and spider plot showing individual subject tumor burden over time.

12.4.2 Analysis Plans for Secondary Objectives

Safety and Tolerability: All adverse events recorded during the trial will be summarized for the safety population. The incidence of events that are new or worsening from the time of first dose of treatment will be summarized according to system organ class and/or preferred term, severity (based on CTCAE version 4.0 grade), type of adverse event, and relation to study treatment. Deaths reportable as SAEs and non-fatal serious adverse events will be listed by subject and tabulated by primary system organ class, and type of adverse event.

PFS and OS: PFS and OS will each be summarized using the product-limit method of Kaplan-Meier. Median times for each endpoint will be presented with two-sided 90% confidence intervals estimated using log(-log(survival)) methodology. Kaplan-Meier estimates of PFS at 6 or 12 months after treatment initiation may also be presented with two-sided 90% confidence intervals.

12.4.3 Analysis Plans for Exploratory Objectives

It's expected that 80% of the trial samples will have adequate specimens for the correlative study and the evaluable samples for the correlative analyses will be 32 (80% of 40 subjects).

A DFCI mutation screen analysis of 300 cancer genes (Oncopanel), including all those involved in regulation of mTOR, will be performed on pre-treatment biopsies in all subjects.

The mutation profiling will be performed using next generation sequencing via Oncopanel in all cases and whole exome sequemcing in a subset of subjects.

The sample size/power justification is focused on the primary objective of assessing the association between ORR and mTOR pathway mutation status (defined as mutation(s) observed in 1 gene or several genes belonging to mTOR pathways). To examine the response (or ORR) by the mutation status (mutated or non-mutated), the evaluable samples will be divided retrospectively according to objective response or non-response. Pre-treatment mutation status will be summarized descriptively for the response/non-response groups.

The trial's targeted ORR rate was 0.2. Based on our experience (submitted for publication) the mutation rate for a cohort of mRCC subjects treated with mTOR inhibitors was 20% (16/79), we assumed a mTOR pathway gene mutations rate of 0.25 for the power estimation, as it is suggested that "23-38% of subjects likely have alteration in the mTOR pathway.

With an evaluable correlative sample size of 32, the study has 80% power to detect a 48% increase (one-sided alpha=0.05) in response probability of 56% in subjects with mutations as compared to that of 8% in subjects without mutations.
The proportion of subjects with objective response according to pre-treatment (baseline) nutation status will also be summarized with two-sided 90% exact binomial CI. Kaplan-Meier estimates will be used to assess the distribution of PFS according to mutation status. Medians of time to PFS will be shown with two-sided 90% CIs.

12.5 Interim Analysis/Criteria for Stopping Study

There are no formal interim efficacy analyses planned for this study.

13. DATA REPORTING/REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 11 (Adverse Events)

13.1 Data Reporting

13.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

13.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality in accordance with DF/HCC SOPs.

13.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a

summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request

13.3 Multicenter Guidelines

This protocol will adhere to the policies and requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan. The specific responsibilities of the Overall PI, Coordinating Center, and Participating Institutions and the procedures for auditing are presented in Appendix E.

- The Overall PI/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

- Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.

- Except in very unusual circumstances, each participating institution will order the study agent(s) directly from supplier. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded to the Coordinating Center.

13.4 Data Quality Oversight Activities

Validation of data will be completed on a continual basis throughout the life cycle of the study. A summary report (QC Report) of these checks together with any queries resulting from manual review of the eCRFs will be generated for each site and transmitted to the site and the site monitor. Corrections will be made by the study site personnel.

There will be at least one routine visit per site per year for sites that have accrued. Additional for cause visits may occur as necessary. Source documents will be reviewed for verification of agreement with data entered into InForm. It is important for the site investigator and their relevant personnel to be available for a sufficient amount of time during the monitoring visits or audit, if applicable. The site investigator and institution guarantee access to source documents by the sponsor/investigator or its designee.

The trial site may also be subject to quality assurance audit by Millennium or its designee as well as inspection by appropriate regulatory agencies.

13.5 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the sponsor-investigator of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. All results of primary and secondary objectives must be posted to CT.gov within a year of

completion. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and study site contact information.

14. DATA HANDLING AND RECORD KEEPING

14.1 Data Management

The Dana-Farber Cancer Clinical Trials Office will provide Project Management and Monitoring services for this trial. Data will be collected through the web based clinical research platform, InForm , a system compliant with Good Clinical Practices and Federal Rules and Regulations created by the Clinical Trials Research Informatics Office (CTRIO). All data will be collected and entered into InForm by study site personnel from participating institutions.

14.2 Case Report Forms and Submission

Generally, clinical data will be electronically captured in InForm and correlative results will be captured in spreadsheets or other secure database(s). If procedures on the study calendar are performed for standard of care, at minimum, that data will be captured in the source document. Select standard of care data will also be captured in InForm, according to study-specific objectives.

The completed dataset is the sole property of the sponsor-investigator's institution and should not be exported to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without permission from the sponsor-investigator .

14.3 Record Retention

To enable evaluations and/or audits from Health Authorities, the site investigator agrees to keep records, including the identity of all subjects (sufficient information to link records; e.g., hospital records), all original signed informed consent forms, copies of all source documents, and detailed records of drug disposition. All source documents are to remain in the subject's file and retained by the site investigator in compliance with the site contract. . No records will be destroyed until the sponsor/investigator confirms destruction is permitted.

14.4 Confidentiality

There is a slight risk of loss of confidentiality of subject information. All records identifying the subjects will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. Information collected will be maintained on secure, password protected electronic systems. Paper files that contain personal information will be kept in locked and secure locations only accessible to the study site personnel.

Subjects will be informed in writing that some organizations including the sponsor-investigator and his/her research associates, Millennium, IRB, or government agencies, like the FDA, may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

15. PUBLICATION PLAN

The data will be collected by the investigators and analyzed by the co-principal investigators and the statistical team at DFCI. It is anticipated that the results will be made public within 12 months of the end of data collection. A report is planned to be published in a peer-reviewed journal, however initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. [redacted] in consultation with [redacted] as well as outside investigators will decide on the authorship orders for abstracts and manuscripts from the trial.

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APPENDIX A: PERFORMANCE STATUS CRITERIA

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

APPENDIX B: REQUIRED FORMS AT REGISTRATION

Please notify the lead clinical research coordinator at the time a participant is identified or consented to the study. The DFCI coordinator will register the participant once the below documentation is finalized using the DFCI OnCore registration system. Non-DF/HCC participating sites will send the documents to the DFCI research coordinator to complete registration. Please allow a one-week turn-a-round time for the DFCI research team to determine participant eligibility. The following documentation is required prior to participant registration:

- Current IRB approved consent form signed by participant and Investigator (MD only)
- HIPAA authorization form (if separate from the informed consent document)
- Signed and dated DFCI eligibility checklist (signed by MD and RN)
- The following source documentation is typically required:
- Please note: Additional documentation may be required by the lead institution.
    - Documentation of prior treatments/procedures performed to treat RCC
    - Reports documenting disease status
        - MRI or CT Brain
        - Chest CT
        - CT or MRI Abdomen and Pelvis
    - Pathology Report
    - Concomitant medication list
    - Progress note or equivalent documentation of consenting visit
    - Progress note documenting medical history and oncologic history
    - Screening Labs
        - Complete blood count with differential
        - Comprehensive Metabolic Profile (CMP)
        - Fasting Lipid Profile
        - PT/INR and aPTT
        - TSH
        - Hb1c
        - On home daily fasting glucose monitoring
        - Pregnancy test
        - Urinalysis
    - Screening visit note with vital signs, weight, height, ECOG performance status, physical examination
    - Screening ECG
    - Screening ECHO or MUGA

PLEASE NOTE: ADDITIONAL DOCUMENTATION MAY BE REQUIRED BY THE LEAD INSTITUTION.

APPENDIX C: INFORMATION ON POSSIBLE DRUG INTERACTIONS

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

List of Relevant Cytochrome P450 Inhibitors and Inducers

Strong CYP2C19 Inhibitors		
Fluconazole	fluvoxamine	ticlopidine
Moderate CYP3A4 Inhibitors		
amprenavir	darunavir/ritonavir	fosamprenavir
Aprepitant	Diltiazem	grapefruit juice (a)
Atazanavir	erythromycin	imatinib
Ciprofloxacin	Fluconazole	verapamil
Strong CYP3A4 Inhibitors		
Boceprevir	ketoconazole	ritonavir
Clarithromycin	lopinavir/ritonavir	saquinavir
Conivaptan	mibefradil (b)	telaprevir
grapefruit juice (a)	nefazodone	telithromycin
Indinavir	Nelfinavir	voriconazole
Itraconazole	posaconazole	
Clinically Significant Enzyme Inducers		
Carbamazepine	Rifabutin	St. Johns Wort
Phenobarbital	Rifampin	
phenytoin	rifapentine	

Note that these lists are not exhaustive.

a. The effect of grapefruit juice varies widely among brands and is concentration-, dose-, and preparation-dependent. Studies have shown that it can be classified as a "strong CYP3A inhibitor" when a certain preparation was used (eg, high dose, double strength) or as a "moderate CYP3A inhibitor" when another preparation was used (eg, low dose, single strength).
b. Withdrawn from the United States market because of safety reasons.

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

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

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

These are the things that you and they need to know:

- TAK-228 interacts with certain specific enzymes in your liver.
- The enzymes in question are CYP2C9, CYP2C19 and CYP3A4, and TAK-228 is broken down by these enzymes in order to be cleared from your system.
- TAK-228 must be used very carefully with other medicines that need these liver enzymes to be effective or to be cleared from your system.
- Other medicines may also affect the activity of the enzyme.
    - Substances that increase the enzyme's activity ("inducers") could reduce the effectiveness of the drug, while substances that decrease the enzyme's activity ("inhibitors") could result in high levels of the active drug, increasing the chance of harmful side effects.
    - TAK-228 is considered a weak "inhibitor" of the enzyme, meaning that it can affect the levels of other drugs that are processed by that enzyme. This can lead to harmful side effects and/or reduce the effectiveness of those medications.
- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drug in this category.
- Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors or substrates of CYP2C9, CYP2C19 and CYP3A4.
- Your prescribers should look at this web site http://medicine.iupui.edu/clinpharm/ddis/table.aspx or consult a medical reference to see if any medicine they want to prescribe is on a list of drugs to avoid.
- Please be very careful! Over-the-counter drugs have a brand name on the label—it's usually big and catches your eye. They also have a generic name—it's usually small and located above or below the brand name, and printed in the ingredient list. Find the generic name and determine, with the pharmacist's help, whether there could be an adverse interaction.
- Be careful:
    - If you take acetaminophen regularly: You should not take more than 4 grams a day if you are an adult or 2.4 grams a day if you are older than 65 years of age. Read labels carefully! Acetaminophen is an ingredient in many medicines for pain, flu, and cold.

- If you drink grapefruit juice or eat grapefruit: Avoid these until the study is over.
- If you take herbal medicine regularly: You should not take St. John's wort while you are taking TAK-228.

Other medicines can be a problem with your study drugs.

- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.
- Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you.

INFORMATION ON POSSIBLE DRUG INTERACTIONS	
You are enrolled on a clinical trial using the experimental agent TAK-228. This clinical trial is sponsored by the NCI. TAK-228 interacts with drugs that are processed by your liver. Because of this, it is very important to: ➢ Tell your doctors if you stop taking regular medicine or if you start taking a new medicine. ➢ Tell all of your prescribers (doctor, physicians' assistant, nurse practitioner, and pharmacist) that you are taking part in a clinical trial. ➢ Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.	TAK-228 interacts with a specific liver enzyme called CYP2C9, CYP2C19 and CYP3A4, and must be used very carefully with other medicines that interact with this enzyme. ➢ Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors or substrates of CYP2C9, CYP2C19 and CYP3A4." ➢ Before prescribing new medicines, your regular prescribers should go to http://medicine.iupui.edu/clinpharm/ddis/table.aspx for a list of drugs to avoid, or contact your study doctor.

APPENDIX D: SUBJECT DIARY

Protocol Number: 16-527

Name: _____ **Date:** _____ **Cycle:** _____

Drug Strength: ___ mg capsules
Dose: ___ mg (# ___ capsules)
Schedule: Once per week in the morning at approximately the same time (e.g. Day 1, 8, 15, and 22)

Dosing Instructions: The TAK-228 capsules will be supplied at the start of each cycle. You will take your doses on an empty stomach in the morning with water. You should not have anything to eat for at least 2 hours before and 1 hour after you take your dose. Please do not crush, chew, dissolve, or open your TAK-228 capsules. If you come into contact with the powder from the capsule, wash your skin thoroughly with soap and water. If you do not take your TAK-228 dose within the time frame specified (± 24 hours of the weekly scheduled dosing time), then the dose should be skipped and considered a missed dose. Resume the dose when your next scheduled dose is due, and mark the dose as missed on your study diary. If you vomit a dose, do not make up the dose, and mark this on your study diary. If you can't remember whether you missed a dose, consider the dose missed, and resume dosing at your next scheduled dose.

On day 1 of each cycle, please bring this completed diary and any unused or empty study bottles to your visit and return it to your study team. If you are having new or worsening symptoms, please contact your study nurse.

At-home Blood Glucose Monitoring Instructions: You will be provided with a kit for at-home blood glucose monitoring. You will be required to measure your daily blood glucose levels while you are on this study. Please measure your glucose level when you are in a fasting state (no food or drink, except water/medication, for at least 8 hours). We recommend you measure your blood glucose level first thing in the morning when you wake up. Please write your blood glucose levels in the spaces provided on your diary below. If any fasting blood glucose levels are greater than or equal to 150 mg/dL please contact your study team as soon as possible. On days when you are scheduled to come into clinic, you will not be required to check your fasting blood glucose level at home beforehand as your study team will be checking it in the clinic.

Page Operator (Holidays, Weekends, and Non-Business Hours):
_____; ask for your site investigator to be paged.

Research Nurse: _____ **Phone:** _____

Day	Date	Time (AM)	Number of Capsules	Fasting blood glucose level	Time (AM)

Day 1*					
Day 2					
Day 3					
Day 4					
Day 5					
Day 6					
Day 7					
Day 8*					
Day 9					
Day 10					
Day 11					
Day 12					
Day 13					
Day 14					
Day 15*					
Day 16					
Day 17					
Day 18					
Day 19					
Day 20					
Day 21					
Day 22*					
Day 23					
Day 24					
Day 25					
Day 26					
Day 27					
Day 28					

*Suggested days to take your dose within a cycle

DFCI IRB Protocol #: 16-527

APPENDIX E

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

1.0 INTRODUCTION

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

1.1 Purpose

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

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

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

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

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

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

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

DF/HCC Office of Data Quality (ODQ): A group within DF/HCC responsible ensuring high-quality standards are used for data collection and the ongoing management of clinical trials, auditing, and data and safety monitoring. ODQ also coordinates quality assurance efforts related to multi-center clinical research.

DF/HCC Clinical Trials Research Informatics Office (CTRIO): A group within DF/HCC responsible for providing a comprehensive data management platform for managing clinical trial data.

2.0 GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the DF/HCC Sponsor, the Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

2.1 DF/HCC Sponsor

The DF/HCC Sponsor, Bradley McGregor, MD, will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

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

2.2 Coordinating Center

The general responsibilities of the Coordinating Center may include but are not limited to:

- Assist in protocol development.
- Maintain FDA correspondence, as applicable.
- Review registration materials for eligibility and register participants from Participating Institutions in the DF/HCC clinical trial management system (CTMS).
- Distribute protocol and informed consent document updates to Participating Institutions as needed.
- Oversee the data collection process from Participating Institutions.
- Maintain documentation of Serious Adverse Event (SAE) reports and deviations/violation submitted by Participating Institutions and provide to the DF/HCC Sponsor for timely review and submission to the DFCI IRB, as necessary.
- Distribute serious adverse events reported to the DF/HCC Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all Participating Institutions.
- Provide Participating Institutions with information regarding DF/HCC requirements that they will be expected to comply with.
- Carry out plan to monitor Participating Institutions either by on-site or remote monitoring.
- Maintain Regulatory documents of all Participating Institutions which includes but is not limited to the following: local IRB approvals/notifications from all Participating Institutions, confirmation of Federal wide Assurances (FWAs) for all sites, all SAE submissions, Screening Logs for all sites, IRB approved consents for all sites
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc.) and maintain documentation all relevant communications

2.3 Participating Institution

Each Participating Institution is expected to comply with all applicable Federal Regulations and DF/HCC requirements, the protocol and HIPAA requirements.

The general responsibilities for each Participating Institution may include but are not limited to:

- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain site regulatory files as per DF/HCC Sponsor/ Coordinating Center requirements.
- Provide the Coordinating Center with regulatory documents or source documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as required (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities.
- Submit Serious Adverse Event (SAE) reports to IRB per local requirements and to the Coordinating Center, in accordance with protocol requirements.

- Submit protocol deviations and violations to IRB per local requirements and in accordance with study requirements.
- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.
- Participate in any quality assurance activities and meet with DFCI monitors or DFCI ODQ auditors at the conclusion of a visit to review findings.
- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.

3.0 DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

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

3.1 Protocol Distribution

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

3.2 Protocol Revisions and Closures

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

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within 90 days from receipt of the notification.
- **Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval.
- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.3 Informed Consent Requirements

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

Participating Institutions are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior

to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB.

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

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

- Initial approval letter of the Participating Institution's IRB.
- Copy of the Informed Consent Form(s) approved by the Participating Institution's IRB.
- Participating Institution's IRB approval for all amendments.
- Annual approval letters by the Participating Institution's IRB.

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

3.5 IRB Re-Approval

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

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

3.6 Participant Confidentiality and Authorization Statement

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

In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an Authorization. This Authorization may or may not be separate from the informed consent document. The Coordinating Center, with the approval from the DFCI IRB.

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

3.6.1 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center should be de-identified. It is recommended that the assigned protocol case number (as described below) be used for all participant specific documents. Participant initials may be included or retained for cross verification of identification.

3.7 DF/HCC Multi-Center Protocol Registration Policy

- Informed consent must be obtained from subjects before they can be screened. A patient will be considered "in screening" when he/she has signed the IRB approved Informed Consent Form.
- Each patient screened must be documented on the Potential Patient/Pre-Screening Log or equivalent site form/software. The Potential Patient Log/Pre-Screening Log is to aid in fulfilling the ICH requirement of maintaining a confidential list of patient names. It should be stored in the site study files.
- Eligibility Criteria Worksheet is provided as a tool for registration.
- Once the investigator verifies the patient is eligible, the lead site (DFCI) will formally register the patient in the DF/HCC Clinical Trail Management System (CTMS) and enter the On Study Date. The sequence number used at screening will remain the same for the remainder of the study to identify the enrolled patient.
- Subjects must be registered prior to starting protocol therapy and begin therapy within five business days of the lead site (DFCI) entering the On Study Date into the DF/HCC Clinical Trail Management System (CTMS).

3.7.1 Participant Registration

All subjects must be registered by the lead site (DFCI) through the DF/HCC Clinical Trail Management System (CTMS) in accordance with section 4 of the protocol. A subject is considered registered when an "On Study" date is entered into OnCore.

- To register a participant, the following documents should be completed by the Participating Institution and maintained in the Participating Institutions' records:
  - Signed informed consent document
  - HIPAA authorization form (if separate from the informed consent document)
  - Other appropriate forms (See Section 4 of the Protocol)

  The Coordinating Center will review the submitted documents in order to verify eligibility and consent. To complete the registration process, the Coordinating Center will:
  - Register the participant on the study with the DF/HCC Clinical Trial Management System (CTMS).
  - Upon receiving confirmation of registration, the Coordinating Center will inform the Participating Institution and provide the study specific participant case number, and, if applicable, assigned treatment and/or dose level

Treatment may not begin without confirmation from the Coordinating Center that the participant has been registered.

Registration can only occur during normal business hours, Monday through Friday from 8:00 AM to 5:00 PM Eastern Standard Time.

3.7.2 Initiation of Therapy

Participants must be registered with the DF/HCC CTMS before the initiation of treatment or other protocol-specific interventions. Treatment and other protocol-specific interventions may not be initiated until the Participating Institution receives confirmation of the participant's registration from the Coordinating Center. The DF/HCC Sponsor and DFCI IRB must be notified of any violations to this policy

3.7.3 Eligibility Exceptions

No exceptions to the eligibility requirements for a protocol without DFCI IRB approval will be permitted. All Participating Institutions are required to fully comply with this requirement.

3.8 DF/HCC Protocol Case Number

At the time of registration, Coordinating Center requires the following identifiers for all subjects: initials, date of birth, gender, race and ethnicity. Once eligibility has been established and the participant successfully registered, the participant is assigned a unique sequence number. Participating Institutions should submit all de-identified subsequent communication and documents to the Coordinating Center, using this sequence number to identify the subject.

3.8.1 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor, who in turn is responsible for reporting to the DFCI IRB.

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

3.8.2 Definitions

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

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

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

3.8.3 Reporting Procedures

DF/HCC Sponsor: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol deviations occurring at their site. The DF/HCC Sponsor will also be responsible for ensuring that all protocol deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from the DFCI IRB. The Participating Institution must submit the deviation request to the Coordinating Center who will then submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation is submitted to the Participating Institution IRB, per institutional policy. A copy of the Participating Institution's IRB report and determination will be forwarded to the Coordinating Center within 10 business days after the original submission. The deviation may not be implemented without all required approvals.

All protocol violations must be sent to the Coordinating Center in a timely manner. The Coordinating Center will provide training for the requirements for the reporting of violations.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines. DF/HCC will forward all violation reports to CTEP via an internal DF/HCC process, as applicable.

3.9 Safety Assessments and Toxicity Monitoring

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

All participants receiving investigational agents and/or other protocol mandated treatment will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol. Life-threatening toxicities must be reported to the DF/HCC Sponsor within 1 business days of becoming aware of the event.

Additional safety assessments and toxicity monitoring is outlined in the protocol.

3.9.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol Section 11.

Participating Institutions must report the SAEs to the Coordinating Center according to the protocol.

The Coordinating Center will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating to all participating investigators, any observations reportable under the protocol requirements. Participating Institutions will review and submit to their IRB according to their institutional policies and procedures

3.9.2 Guidelines for Processing IND Safety Reports

The DF/HCC Sponsor will review all IND Safety Reports and ensure that all IND Safety Reports are distributed to the Participating Institutions. Participating Institutions will review and submit to their IRB according to their institutional policies and procedures.

3.10 Data Management

Data will be handled and recorded in accordance with Section 14 of the protocol.

3.10.1 Data Forms Review

Data submissions are monitored for timeliness and completeness of submission. If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC Office of Data Quality, Coordinating Center, or designee.

Responses to all queries should be completed and submitted within 14 calendar days.

Responses may be returned on the written query or on an amended paper case report form, or in the case of electronic queries, within the electronic data capture (eDC) system. In the case of a written query for data submitted on a paper case report form, the query must be attached to the specific data being re-submitted in response.

If study forms are not submitted on schedule, the Participating Institution will periodically receive a Missing Form Report from the Coordinating Center noting the missing forms.

4.0 REQUISITIONING INVESTIGATIONAL DRUG

Investigational Drug (TAK-228) will be provided by Millennium Pharmaceuticals.

5.0 MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires verification of protocol compliance and data accuracy. The Coordinating Center, with the aid of the DF/HCC Office of Data Quality, provides quality control oversight for the protocol.

5.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions may be required to submit participant source documents to the Coordinating Center for monitoring. Participating Institutions may also be subject to on-site monitoring conducted by the Coordinating Center.

DFCI will implement ongoing monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring will occur during protocol performance and through study completion. Additional monitoring practices may include but are not limited to; source verification, review and analysis of the following: eligibility requirements, informed consent procedures, adverse events and all associated documentation, study drug administration/treatment, regulatory files, protocol departures, pharmacy records, response assessments, and data management.

Participating institutions will be required to participate in monthly Coordinating Center initiated teleconferences.

Remote Monitoring
Data will be monitored remotely throughout the study both manually and electronically. Data will be assessed for completeness and correctness by DFCI monitoring staff. A Missing Forms Report (MFR) will be run on a regular basis, and queries will be issued based on the results to site personnel. Site personnel will make all corrections and/or notations as appropriate.

On-Site Monitoring will occur per section 13 of the protocol

5.2 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports to ensure protocol compliance. The DF/HCC Sponsor may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations.

5.3 Accrual Monitoring

Prior to extending a protocol to an external site, the DF/HCC Sponsor will establish accrual requirements for each participating institution. Accrual will be monitored for each participating institution by the DF/HCC Sponsor or designee. Sites that are not meeting their accrual expectations may be subject to termination.

6.0 AUDITING: QUALITY ASSURANCE

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

6.1 DF/HCC Internal Audits

All Participating Institutions are subject to audit by the DF/HCC Office of Data Quality (ODQ). Typically, approximately 3-4 participants would be audited at the site over a 2 day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

6.1.1 Audit Notifications

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

6.1.2 Audit Reports

The DF/HCC Sponsor will review all final audit reports and corrective action plans, if applicable. The Coordinating Center, must forward any reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

6.2 Participating Institution Performance

The DF/HCC Sponsor, and DFCI IRB, is charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

Participating Institutions that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the DF/HCC Sponsor for revocation of participation. A DF/HCC Sponsor and/or the DFCI IRB may terminate a site's participation if it is determined that a site is not fulfilling its responsibilities as described above.