SUMMARY OF CHANGES - Protocol

For Protocol Amendment to: A Phase 2 Study of MLN0128 (TAK-228) in Patients with Advanced Non-Small Lung Cancers Harboring *NFE2L2* and *KEAP-1* Mutations

NCI Protocol #: 9780
Local Protocol #: 15-249

NCI Version Date: 05/14/2020
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| # | Page(s) | Change |
|---|---|---|
| 1. | Throughout | Editorial, formatting and grammatical changes were made throughout the protocol. |
| 2. | Throughout | NCI Version Date has been updated to 05/14/2020 throughout the protocol. |
| 3. | 4-8 | The following clinicians have been added as co-investigators:<br><br>Andrew Chow, MD, PhD<br>Adam Schoenfeld, MD<br>Stuart Lichtman, MD<br>Neil Shah, MBBS<br>Andrew Laccetti, MD, MS<br>Julia Brockway-Marchello, MD<br><br>The following clinicians have been removed as co-investigators:<br><br>Han Xiao, MD<br>Sree Chalasani, MD<br>Parisa Momtaz, MD |

**NCI Protocol #:** 9780

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**ClinicalTrials.gov Identifier**: NCT02417701

**TITLE:** A Phase 2 Study of MLN0128 (TAK-228) in Patients with Advanced Non-Small Cell Lung Cancers Harboring *NFE2L2* and *KEAP1* Mutations

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**Protocol Type / Version # /Version Date:** Amended/Version #16/ Version Date: 06/08/2018
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**Protocol Type / Version # /Version Date:** Amended/Version #20/ Version Date: 05/14/2020

**SCHEMA**

| | |
|---|---|
| **Study Title:** | A Phase 2 Study of MLN0128 (TAK-228) in Patients with Advanced Non-Small Cell Lung Cancers Harboring NFE2L2 and *KEAP-1* Mutations |
| **Study Objectives:** | **Primary Objective:**<br>• Evaluate the overall response rate of the TORC1/TORC2 inhibitor MLN0128 (TAK-228) in *NFE2L2* or *KEAP1* mutant Stage IV squamous cell lung cancers and *KRAS* mutant lung cancers harboring *KEAP1* or *NFE2L2* alterations.<br>**Secondary Objectives:**<br>• To evaluate median progression free survival<br>• Explore the feasibility of performing reverse phase protein array analysis (RPPA) in paired snap-frozen core biopsies from patients in this study prior to MLN0128 (TAK-228) dosing and 1 week afterwards.<br>• Describe the effectiveness of MLN0128 (TAK-228) in suppressing activation of mTOR and PI3K signaling through the exploratory RPPA analysis. |
| **Patient population:** | Stage IV or recurrent squamous cell lung cancer or *KRAS* mutant lung cancer patients who have progressed after at least first-line chemotherapy |
| **Number of patients:** | 40 patients |
| **Inclusion Criteria:** | 1. Refer to Section 3.1 of the protocol |
| **Exclusion Criteria:** | 1. Refer to Section 3.2 of the protocol |
| **Study Drug:** | MLN0128 (TAK-228) |

| | |
|---|---|
| **Study Design:** | This protocol is a phase 2, single institution study of MLN0128 (TAK-228) in patients with stage IV or recurrent squamous cell lung cancer or *KRAS* mutant lung cancer harboring either an *NFE2L2* or *KEAP1* mutation who have progressed after at least first-line chemotherapy.<br><br>The study will enroll three cohorts: 1) squamous cell lung cancer with *NFE2L2* mutations; 2) squamous cell lung cancer with *KEAP1* mutations; 3) *KRAS* mutant lung cancer with concomitant *NFE2L2* or *KEAP1* aberration. A Simon two- stage design will be used to assess the primary endpoint of radiographic objective response rate, with a 5% undesirable response rate (H0) and a 40% ideal response rate (HA) ($\alpha$=0.1, power=90%), for each cohort. Five patients in each cohort will be enrolled into the first stage of the design. If $\geq$1 patient in any cohort develops a response, then an additional 5 patients will be added for the second stage. $\geq$2 patients must respond out of the final cohort of 10 patients to consider MLN0128 (TAK-228) worthy of further investigation.<br><br>Patients will be treated as follows: MLN0128 (TAK-228) 3mg p.o. daily on a continuous schedule.<br><br>Toxicity will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.0. Response to therapy will be assessed by interval imaging with CT scan of the chest after every 2 cycles with response evaluated per RECIST 1.1. |
| **Correlative Studies** | Molecular testing through MSK-IMPACT will be performed to identify *KRAS* mutations and both *NFE2L2* and *KEAP1* mutations as well as somatic changes in hundreds of other oncogenes and tumor suppressors.<br><br>Reverse phase protein array analysis will be performed on optional paired tumor biopsies obtained before treatment begins and during week 2 of treatment. |

**TABLE OF CONTENTS**

## 1. OBJECTIVES

### 1.1 Primary Objectives

1. Evaluate the overall response rate of the TORC1/TORC2 inhibitor MLN0128 (TAK-228) in Stage IV squamous cell lung cancers or *KRAS* mutant lung cancers harboring *NFE2L2* or *KEAP1* mutations

### 1.2 Secondary Objectives

1. To evaluate the median progression free survival of patients in each cohort
2. To explore the feasibility of performing reverse phase protein array analysis (RPPA) in paired snap-frozen core biopsies from patients in this study prior to MLN0128 (TAK-228) dosing and during week 2 of treatment.
3. To describe the effectiveness of MLN0128 (TAK-228) in suppressing activation of mTOR and PI3K signaling through the exploratory RPPA analysis.

## 2. BACKGROUND

### 2.1 Squamous cell lung cancer and NFE2L2/KEAP1 mutations

Non-small cell lung cancer remains the leading cause of cancer related mortality in the US and worldwide. Squamous cell lung cancer accounts for approximately 25% of all non-small cell lung cancer cases. In the United States, 55,000 patients are diagnosed with squamous cell lung cancer per year, with approximately half of these patients harboring metastatic disease at diagnosis. Patients with advanced squamous cell lung cancers generally have a poor prognosis with a median survival of 12 months. Furthermore, these patients have fewer therapeutic options than those with other histologies. Pemetrexed, which has greater efficacy in non-squamous NSCLC, is not FDA approved for patients with squamous cell lung cancers. Because of rare cases of life threatening hemoptysis in early phase clinical trials, bevacizumab is similarly not FDA approved for patients with squamous cell lung cancers. Most pressing of all is the current absence of druggable molecular targets in squamous cell lung cancers. For instance, activating mutations in EGFR that render lung tumors sensitive to erlotinib and afatinib and ALK rearrangements that sensitize to treatment with crizotinib do not occur in SQCLCs.[1,2] Taken together, there remains a high unmet medical need for new treatments for squamous cell lung cancer.

Nrf2, the gene product of *NFE2L2*, is a transcription factor that binds to antioxidant response elements (AREs) contained in the promoters of a number of antioxidant genes and drug efflux pumps. Keap1, the product of *KEAP1*, sequesters Nrf2 to the cytoplasm and is an adapter for the Cul3-dependent E3 ubiquitin ligase, which targets Nrf2 for degradation.[3] During times of oxidative stress, cysteine residues are modified on Keap1, leading to a structural change that releases Nrf2 for nuclear translocation. *Nrf2-/-* mice are susceptible to carcinoma formation in

response to chemical mutagens, notably benzo[a]pyrene, which is a carcinogen found in tobacco smoke. In patients with SQCLCs, *NFE2L2* mutations occur exclusively in a single N-terminal region coined Neh2, which is the canonical Keap1 binding domain (aa.1-80). Mutations in *KEAP1* are sporadic but occur in functional domains; 50% occur in the C-terminal Nrf2 binding domains; the other 50% occur in the N-terminal BTB and IVR domains that regulate Nrf2 activity. Collectively, these genes are mutated in ~20-30% of SQCLC patients, circumscribing one of the largest SQCLC subtypes described to date.

Shibata et al. previously characterized the oncogenic potential of activating mutations in *NFE2L2*. HEK293 cells expressing Neh2-domain mutant *NFE2L2* cDNAs exhibit increased cell proliferation and colony formation and develop sporadic tumors in orthotopic mouse models (Figure 1 and 2).[4] These tumors were universally poorly differentiated carcinomas with high mitotic activity and a propensity for local invasion, although metastases were also observed. Signaling experiments demonstrated an upregulation in S6 kinase activity and not MAPK, which was supported by gene set enrichment analysis of *NFE2L2* mutant cells that identified mTOR as a significantly upregulated pathway. Further experiments isolated a small G protein, RagD, as the transcriptional target of Nrf2 which induces activation of mTOR signaling. More recent data from Bendavit and colleagues has also shown that Nrf2 can bind directly to the MTOR promoter sequence.[6]

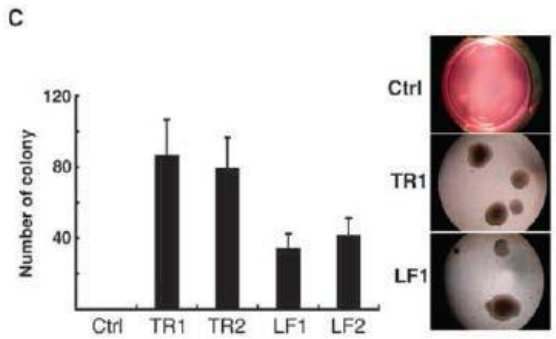



**Figure 1**. Increased anchorage independent growth in HEK293 cells expressing mutant Nrf2. Ctrl=wild type Nrf2; TR1/TR2=Nrf2 T80R; LF1/LF2=Nrf2 L30F.

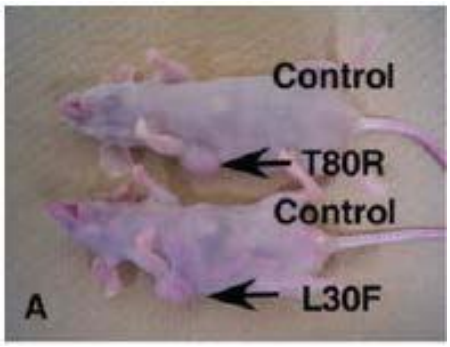



**Figure 2**. Spontaneous tumor formation in *Nrf2* mutant xenograft models. Ctrl=wild type Nrf2; T80R=Nrf2 T80R clone; L30F=Nrf2 L30F clone.

Importantly, both rapamycin and BEZ235 (a PI3K/TORC1 inhibitor) were cytotoxic to two squamous cell lung cancer cell lines (LK2 and EBC1) that endogenously harbor canonical (Neh2 domain) *NFE2L2* mutations but not WT cell lines (Figure 3). BEZ235 also abolished tumor growth in an associated xenograft model of LK2 SQCLC cells (Figure 4). While Nrf2 signaling has not been a formal focus of the MLN0128 (TAK-228) clinical development program, it is worth noting that of the 5 human tumor cell line xenografts in which MLN0128 (TAK-228) was found to be effective, one, the *KRAS*-mutant A549 NSCLC cell line, also harbors a *KEAP1* G333C mutation (which occurs in one of the 6 Nrf2-binding Kelch domains) and exhibits *KEAP1* loss of heterozygosity (loss of 19p13.2).[3] A549 cells are also associated with increased Nrf2 nuclear localization compared to *KEAP1* WT cells. The specific *KEAP1* G333C mutation is also unable to abolish Nrf2-mediated ARE reporter activity, supporting its functional impact in A549 cells.

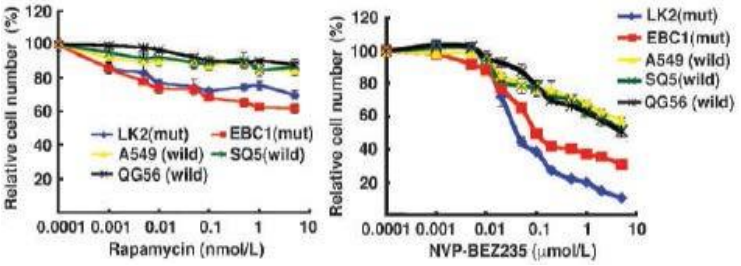



**Figure 3**. Decreased cell viability in Nrf2 mutant squamous cell lung cancer cell lines (LK2 and EBC1) treated with rapamycin and the PI3K/mTOR inhibitor BEZ235.

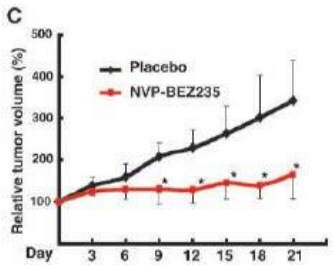



**Figure 4**. Inhibition of tumor growth in Nrf2 mutant LK2 mouse xenografts treated with BEZ235.

## 2.2 KRAS mutant lung cancers harboring alterations in the *NFE2L2/KEAP1* pathway

In keeping with the observation that the *KRAS* mutant A549 NSCLC cell line harboring *KEAP1* loss of heterozygosity is associated with increased Nrf2 nuclear localization, recent data from Romero and colleagues[5] have elucidated a role for Nrf2 activation in *KRAS* mutant lung cancer. In sum, they showed that *KEAP1* loss in a KRAS-driven lung cancer model hyperactivates Nrf2 leading to resistance to multiple oxidative stress agents. This phenotype could be rescued by the addition of antioxidant treatments or exogenous reconstitution of Nrf2. Loss of *KEAP1* also accelerated tumorigenesis and growth (**Figure 5A-D**). A CRISPR-Cas9-based genetic screen further identified dependence on the glutamine transporter SLC1A5. Taken together, these data suggest that *KRAS* mutant lung cancer harboring events that activate Nrf2 via *NFE2L2* or *KEAP1* alterations would also be rational targets for inhibition of this pathway. Pre-clinical data generated by us in Section 2.3 demonstrates that TAK228 monotherapy does indeed have anti-tumor efficacy in this context. Existing data published by TCGA and us (through MSK-IMPACT) shows that the proportion of *KRAS* mutant lung cancer patients who tumors harbor concomitant alterations in *KEAP1* or *NFE2L2* is relatively high, at 25.6%. Of the 405 patients with *KRAS* mutant lung cancers sequenced at MSK since 2014, 102 patients had *KEAP1* alterations, 25% of which were nonsense or frameshift deletion mutations. 2.22% had canonical mutations in the *NFE2L2* Neh2 domain.

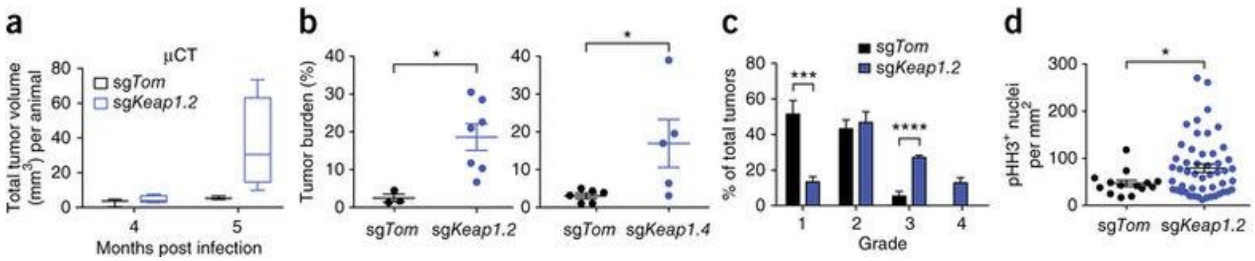



**Figure 5** (a) Micro-CT quantification of total tumor volume (mm3) for tumors from sgKeap1.4 or sgTom mice (n = 5 and 3, respectively) at 4 and 5 months after infection. Whiskers represent minimum and maximum values, upper and lower perimeters represent the interquartile distance, and midlines represent mean values. (b) Combined quantification of tumor burden (total tumor area/total lung area) in KP mice after infection with pSECC lentiviruses. Left, tumor burden of mice infected with control sgTom (n = 3) or sgKeap1.2 (n = 7) at 21 weeks after infection. Right, tumor burden in mice infected with control sgTom (n = 6) or sgKeap1.4 (n = 5) at 21 weeks after infection. Asterisks indicate statistical significance obtained from comparing KP-sgKeap1 samples to KP-sgTom samples. (c) Distribution of histological tumor grades in KP mice 21 weeks after infection with pSECC lentiviruses expressing control (sgTom KP, n = 7 mice) or sgKeap1.2 (KP; n = 3 mice). (d) Quantification of phosphorylated histone H3 (pHH3)-positive nuclei per squared millimeter of tumor for assessment of the mitotic index of tumor cells from lung tumors in KP mice at 21 weeks after infection with pSECC lentiviruses expressing control (sgTom; n = 14 tumors) or sgKeap1.2 (n = 50 tumors).

## 2.3 MLN0128 (TAK-228)

Mechanism of Action

MLN0128 (TAK-228) is an orally available inhibitor that has demonstrated potent and selective inhibition of mTOR kinase (Investigator's Brochure, 2012). MLN0128 (TAK-228) exerts a dual mechanism of action targeting both TORC1 and TORC2 (TORC1/2) complexes. MLN0128 (TAK-228) is in the category of ATP-competitive inhibitors, as it competes with ATP for binding to TORC1/2 active sites.

*In vitro* antitumor activity

MLN0128 (TAK-228) selectively and potently inhibited mTOR kinase, demonstrating 50% inhibition at the concentration of 1.1 nM ($IC_{50}$). Relative to mTOR, MLN0128 (TAK-228) was >100-fold less potent as an inhibitor of Class I (PI3 kinase isoforms α, β, γ, δ (Table 1), class II (PI3KC2α and PI3K2Cβ), and Class III (VPS34) PI3K family members, as well as PI3Kα and PI3Kβ.

***Table 1. Comparison of MLN0128 (TAK-228) inhibition power against mTOR and a family of PI3 kinases***

| ***Recombinant Enzyme*** | ***$IC_{50}$ (nM)*** |
|---|---|
| *PI3Kα* | *254* |
| *PI3Kβ* | *4183* |
| *PI3Kγ* | *221* |
| *PI3Kδ* | *223* |
| *mTOR* | *1.1* |

*$IC_{50}$: Drug concentration at which 50% inhibition is achieved (values represent the averages of 16-25 repeat experiment*

MLN0128 (TAK-228) (1 mcM) also inhibited (>80%) biochemical activity of five kinases (mTOR, DNA-

PK, PDGFRα, Flt3, and CK1 epsilon kinases) out of a panel of 222 protein kinases. MLN0128 (TAK-228) inhibited ligand binding of 10 receptor and intracellular protein kinases including (ACVR1, BMPR1B, CSF1R, CSNK1D, CSNK1E, DDR1, MEK1, MEK2, PDGFRβ, and RIPK2) out of a panel of 402 distinct kinases. MLN0128 (TAK-228) displayed cellular inhibition of TORC1 and TORC2 pathways with $IC_{50}$ less than 10 nM.

In Vivo Studies:

Pharmacokinetic (PK) studies of MLN0128 (TAK-228) have been investigated in a number of animal models, including mice, rats, dogs, and monkeys. A summary of the non-clinical PK are shown in Table 4-3 from the Investigator's Brochure v.7, below.

**Table 4-3 Plasma Pharmacokinetics of MLN0128 in Various Species After Intravenous Dosing**

| Species | Dose (mg/kg) | $t_{1/2}$ (hr) | CL (mL/min/kg) | $V_{ss}$ (L/kg) |
|---|---|---|---|---|
| Male BALB/c mice | 2 | 1.1 | 30.6 | 2.8 |
| Male Sprague-Dawley rats | 2.25 | 6.4 | 27.6 | 14.3 |
| Male beagle dogs | 1 | 18.9 | 0.9 | 1.4 |
| Male cynomolgus monkeys | 1 | 6.8 | 2.9 | 1.7 |

Source: Reports ADME-09-012, ADME-09-008, ADME-09-005, and ADME-09-003.

Abbreviations: CL = clearance; $t_{1/2}$ = half-life; $V_{ss}$ = estimated steady-state volume of distribution.

Note: MLN0128 was formulated as an aqueous solution containing 30% polyethylene glycol 400 (PEG400) and 5% to 10% cosolvent and surfactant for intravenous administration.

Drug Metabolism and Pharmacokinetics: TAK-228 was rapidly absorbed after PO administration to mice, rats, dogs, and monkeys,with high oral bioavailability. 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 did not inhibit P-glycoprotein, but did inhibit breast cancer-resistance protein (BCRP), organic cation transporter (OCT)1 and OCT2.

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.

Toxicology: Adverse events of TAK-228 in rats and monkeys included body weight loss, decreased activity, increased glucose and insulin levels, alterations in white blood cells, bone marrow and lymphoid depletion, thymic necrosis, oligospermia, testes degeneration/atrophy, nonglandular stomach epithelial degeneration/ulceration/hyperplasia, pancreatic islet degeneration and fibrosis, lens fiber degeneration with cataract correlate, adrenal cortex hypertrophy, pituitary atrophy secondary to body weight loss, liver

hepatocellular vacuolation, retinal dysplasia with or without optic nerve atrophy, and alveolar histiocytosis. TAK-228 was negative for genotoxicity in an in vitro bacterial mutagenesis (Ames) assay, an in vivo rat micronucleus assay, and an in vivo rat comet assay. TAK-228 was negative for phototoxicity in the 3T3 fibroblast assay.

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

Clinical Development of MLN0128 (TAK-228)

The safety and tolerability of single-agent MLN0128 (TAK-228) (TAK-228) has been evaluated in two phase 1 company-sponsored studies: 1) the first-in-human dose-finding study in patients with advanced solid malignancies (Study INK128-001; n=166) and 2) a dose-finding study in patients with relapsed or refractory MM and Waldenstrom macroglobulinemia (WM), and non- Hodgkin's Lymphoma (NHL) (Study INK128-002; n=39). MLN0128 (TAK-228) (TAK-228) has been administered on a 28-day cycle via various dosing schedules, including the continuous daily (once a day; QD) regimen or the intermittent regimens, such as the once weekly (QW), the 3-day-on/4-day-off repeated weekly (QD×3d every week), and the 5-day-on/2-day-off repeated every week (QDx5d every week) schedules. In addition, the company has initiated a phase 1b combination study of MLN0128 (TAK-228) with paclitaxel (± trastuzumab in patients with HER2+ and HER2- breast cancer) with advanced solid tumors (INK128-003; n=69).

In the daily-dosing cohort of INK128-001, a total of 25 patients received dose-escalated MLN0128 (TAK-228) daily using a standard 3+3 design. The four cohorts included 2 mg (n=3), 4 mg (n=7), 6 mg (n=7), and 7 mg (n=8). The MTD was 6 mg QD with a DLT of G3 rash in 1 out of 6 patients (Infante *et al.*, AACR 2012). The MTD for the QDx3d QW dosing was 16mg; for the QDx5d QW dosing, 10mg; and for the QW dosing, 40mg. The recommended phase 2 dose of 3mg po qd is based on the reported MTD of 6mg po qd.

Treatment-emergent serious adverse events (SAEs) were reported in 58 patients (41%) in INK128-001. The most common AEs were mucosal inflammation in 5, asthenia and pneumonia in 4, and abdominal pain, stomatitis, and renal failure in 3 each. Most AEs were grade 1 and 2 and manageable with supportive care and/or dose interruption/reduction. Grade ≥ 3 events included hyperglycemia (13%), asthenia (8%), anemia (7%), and hypophosphatemia and lymphopenia (6%). Of 142 patients treated in INK128-001, 20% discontinued due to AEs. As of December 2013, 4 patients had died within 30 days of the last dose of drug, 3 from disease progression and 1 from ventricular fibrillation and cardiac arrest possibly related to study drug.

Summary of Effects in Humans:

TAK-228 has been studied in 2 different phase I clinical trials in patients with solid malignancies and an additional phase I trial in patients with hematologic malignancies utilizing different dosing schedules (QD, QW, QDx3d every week and QDx5d every week) in 28-day cycles *(Infante et al., 2012; Ghobrial et al., 2012; Tabernero et al., 2012). The original trials with TAK-228 used the original unmilled TAK-228 active*

*pharmaceutical ingredient (API); current manufacturing process produces milled TAK-228 API.* The recommended phase 2 dose for unmilled TAK-228 for the daily dosing schedule of the unmilled formulation were determined at 5 mg/day and for a weekly dosing schedule at 30 mg/week. *(Infante et al., 2012; Ghobrial et al., 2012).* A dose limiting toxicity of grade 3 rash was reported in in these trials *(Infante et al., 2012).*

Safety: As of the clinical data cutoff (09 December 2014), a total of 335 patients had received ≥ 1 dose of study drug across studies. A total of 18 deaths that occurred within 30 days of the last study drug dose had been reported to the clinical database as of the data cutoff; of these events, 1 (cardiac arrest; Study INK128-001) was considered related to TAK-228.
At least 1 treatment-emergent SAE, regardless of causality, had been reported in 125/335 patients (37%). Across the studies and regardless of causality or dosing regimen, the most common TEAEs included nausea, fatigue, hyperglycemia, vomiting, diarrhea, stomatitis, and decreased appetite.

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

Clinical Pharmacokinetics

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

The PK of MLN0128 (TAK-228) has been evaluated in over 130 patients in ongoing studies. The mean steady-state plasma $C_{max}$ ranged from 52-232 nM. No significant change in the PK parameters of MLN0128 (TAK-228) was observed on repeat dosing; the mean accumulation index of MLN0128 (TAK-228) ranged from 0.7-1.7-fold following multiple doses. PK parameters in 25 patients with advanced solid tumors receiving MLN0128 (TAK-228) on the QD dosing are presented in Table 5.4 from the Investigator's Brochure v.7.

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

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

Consistent with the QD dosing, MLN0128 (TAK-228) displayed dose-dependent PK on all three intermittent dosing regimens, with rapid absorption ($T_{max}$ ranging from 0.5-4 hours) and mean plasma $t_{1/2}$ 8 hours (Tabernero *et al.*, AACR 2012).

Drug-drug interactions

Multiple human metabolizing enzymes are involved in the Phase I metabolism of MLN0128 (TAK-228). When normalized for human liver content, the CYP isoforms CYP3A4, CYP2C9, and CYP2C19 appear to contribute to MLN0128 (TAK-228) metabolism. MLN0128 (TAK-228) displayed low potential (IC50 > 25 μM) for inhibition of the major human CYP isoforms.

Recently completed in vitro metabolism experiments in human hepatocytes using $^{14}$C-labeled TAK-228 suggest that TAK-228 is metabolized primarily via CYP1A2 (approximately 31%-40%), with a minor contribution from CYP3A4 (approximately 11%-22%). These data suggest that TAK-228 is also metabolized by direct glucuronidation (approximately 22%) and an unidentified non-uridine diphosphate glucuronosyl transferase pathway (approximately 18%). The new data differ from the previous in vitro CYP phenotyping data obtained using recombinant CYP enzymes, which suggested the involvement of CYP2C9 (approximately 35%), CYP2C19 (approximately 28%), and CYP3A4 (approximately 28%) in TAK-228 metabolism. In addition, physiologically based PK modeling and simulation using the new metabolism data for TAK-228 suggest that the risk for a metabolism-based drug-drug interaction with TAK-228 appears to be low. Therefore, strong CYP1A2 inhibitors and CYP inducers should be administered with caution and at the discretion of the investigator during the study.

~~Development of a Milled Formulation of TAK-228~~

~~In order to allow more predictable absorption of TAK-228 after oral administration and to allow scale-up manufacturing of TAK-228 capsules, Millenium/Takeda developed a new milled formulation of the agent. The physical milling step during the granulation process controls particle size distribution of TAK-228. In~~

~~order to observe whether this milling step altered the safety and PK profile of TAK-228, the company performed in vivo studies with PK analysis of milled TAK-228. These studies indicated that the milled formulation may result in faster absorption with possibly higher maximum concentration (Cmax), which could result in a different safety profile, compared to the previous unmilled API capsules.~~

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

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

~~The significant difference in tolerability observed in the comparison of the MTDs between unmilled and milled TAK-228 when administered QD may be possibly explained due to the effect of food on the safety/tolerability of unmilled TAK-228 in study IND128-001. The GastroPlus™ simulation performed under fasting conditions on the trial demonstrated that unmilled and milled TAK-228 administration result in comparable exposures to TAK-228; whereas in the fed state, milled TAK-228 resulted in higher $C_{max}$ (1.5- to 2-fold higher) and earlier $T_{max}$ than unmilled TAK-228 with comparable AUCs. Consequently, a dose of 3 mg QD was chosen as the RP2D of milled TAK-228 dose in empty stomach conditions.~~

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

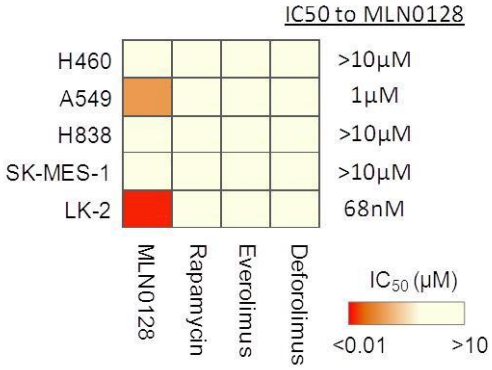
~~TAK-228-1004, a phase I, open label study to evaluate the safety, tolerability, and pharmacokinetics of TAK-228 in combination with paclitaxel in adult patients with advanced non-hematological malignancies ), with the new milled API was used to determine the recommended phase 2 dose (RP2D) for TAK-228 QD×3days per week in combination with paclitaxel. The RP2D of milled TAK-228, given 3 consecutive days weekly, in combination with weekly paclitaxel at 80 mg/m2, was 6 mg.~~

## 2.4 Rationale

While the existing data suggest that TORC1 inhibition may modestly inhibit Nrf2 signaling and cell viability in *NFE2L2* mutant models, the activation of concurrent Akt signaling in *NFE2L2* mutant cell lines, increased efficacy of BEZ235 in the above pre-clinical models, as well the prior experimental data demonstrating a bypass pathway through TORC2/Akt supports the use of dual TORC1/TORC2 inhibition.

To strengthen the rationale for the use of MLN0128 (TAK-228) in squamous lung cancers harboring *NFE2L2* and *KEAP1* mutations, we performed cytotoxicity and signaling experiments in both *NFE2L2* mutant (LK-2) and *KEAP1* mutant (H460, A549, H838) lung cancer cell lines treated with MLN0128

(TAK-228) and a number of rapalogs. We note that while LK-2 cells are squamous cell in histology, all *KEAP1* mutant cell lines were either adenocarcinomas or large cell carcinomas (no *KEAP1* mutant squamous cell lung cancer cell line exists to our knowledge). An Alamar blue assay was used to assess the effect of MLN0128 (TAK-228), rapamycin, everolimus, and deforolimus on cell viability in a dose response manner as shown below in
**Figure 5**.




| Cell Line | *KEAP1* mutation | *NFE2L2* mutation | *KEAP1* CNV | Co-mutations |
|---|---|---|---|---|
| H460 | D236H | WT | diploid | KRAS Q61H, PIK3CA E545K |
| A549 | G333C | WT | loss | KRAS G12S |
| H838 | E444* | WT | diploid | N/A |
| SK-MES-1 | WT | WT | diploid | N/A |
| LK-2 | WT | E79K | N/A | N/A |

**Figure 5**. **Left-** Heatmap of IC50 values for cytotoxicity using Alamar blue. 3 days after seeding, cells were exposed to Alamar blue for 24 hours prior to fixation and readout. IC50 for MLN0128 is shown. IC50s for all rapalogs were ≥10μM for all cell lines tested. **Right-** Table lists genotype data for the cell lines used.

No cytotoxic effect was observed for any of the rapalogs tested against any cell line at any drug concentration. Conversely, MLN0128 (TAK-228) was associated with a low nanomolar IC50 for cytotoxicity in *NFE2L2* mutant LK2 cells and a relatively low IC50 for the *KEAP1* null (*KEAP1* G333C mutant + *KEAP1* loss) A549 cells. Squamous cell lung cancer SK-MES-1 cells WT for *NFE2L2* and *KEAP1* showed no response to MLN0128 (TAK-228). With regard to the differential response in A549 cells compared to the two other *KEAP1* mutant cell lines tested, loss of heterozygosity may potentiate response to drug. It is worth reiterating that A549 cells were found to be sensitive to MLN0128 (TAK-228) by Millenium in their pre-clinical data as well. Alternatively, not all mutations in *KEAP1* may predict for sensitivity to drug inhibition.

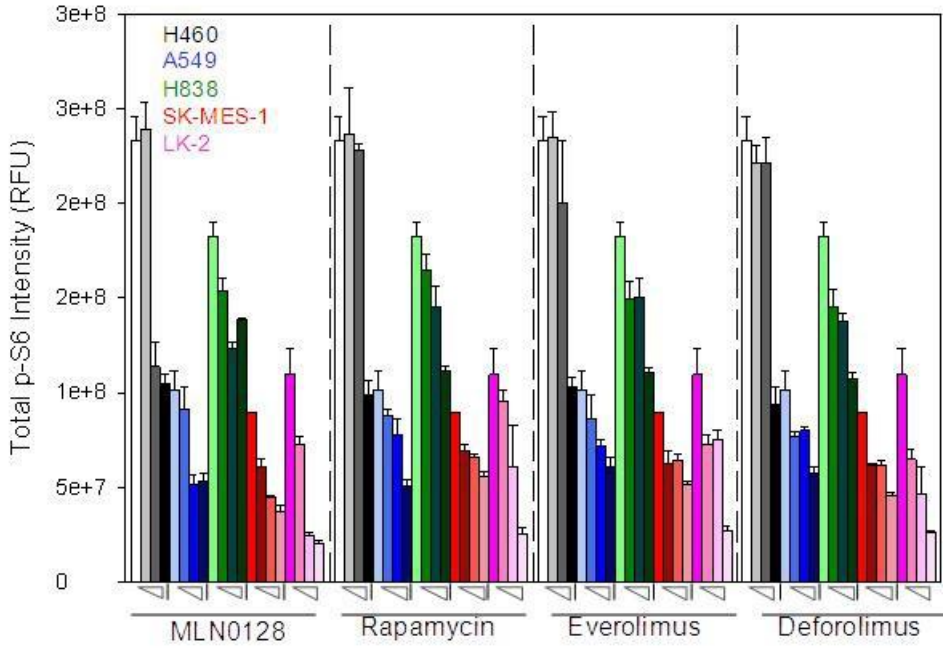
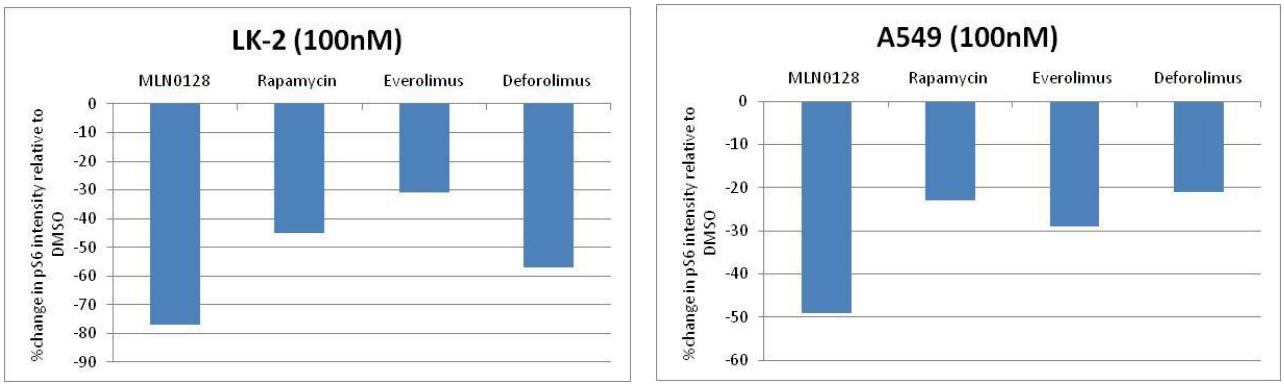



**Figure 6**. Bar graph plot of total phospho-S6 (Ser240/244) staining intensity. Cell lines are represented by color scale: H460 in gray, A549 in blue, H838 in green, SK-MES-1 in red, and LK-2 in pink. Increasing drug concentration is symbolized by the triangles (0, 0.001, 0.1 and 10 μM dose levels)

To determine the downstream effect of each drug on mTOR signaling, cells were fixed and incubated with a phospho-S6 (Ser240/244) antibody after 2 hours of drug exposure. As seen in Figure 6, a dose-dependent decrease in S6 phosphorylation was generally seen across the cell lines in response to each drug. Importantly, inhibition of S6 phosphorylation in LK-2 and A549 cells was substantially greater in response to MLN0128 (TAK-228) compared to the rapalogs at 100nM concentrations (Figure 7 and 8).




**Figures 7 and 8**. Bar graph plot of relative phospho-S6 (Ser240/244) staining intensity at 100nM drug concentrations in *NFE2L2* mutant LK-2 cells (left) and *KEAP1*mutant A549 cells (right). Data are shown relative to DMSO control.

Taken together, these data suggest that MLN0128 (TAK-228) will be superior to the TORC1 rapalogs at inhibiting downstream mTOR signaling and at inducing cell death in *NFE2L2* mutant and a subset of *KEAP1* mutant lung cancers, providing strong rationale for its use in patients with these oncogenic genotypes.

Cell line xenograft studies indeed bear this out in both our *NFE2L2* mutant squamous cell lung cancer LK-2) and *KRAS* mutant/*KEAP1* inactivated (A549) contexts **(Figure 9 and Figure 10)** where MLN0128 (TAK-228) treatment leads to a decrease in the size of generated tumors.

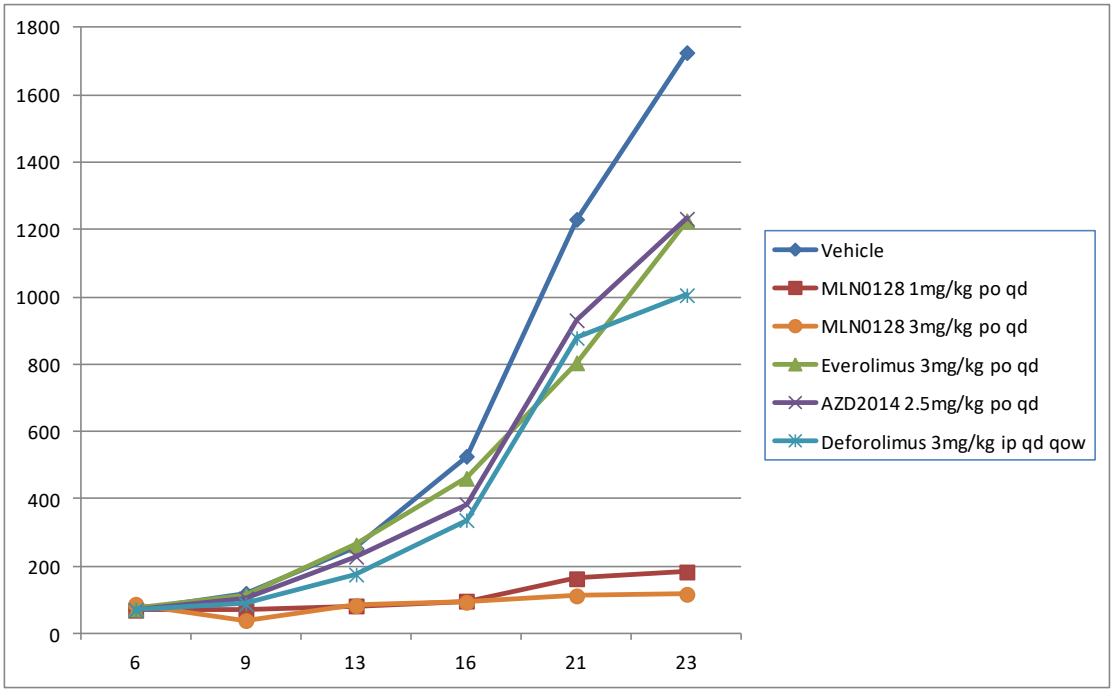



**Figure 9.** Differential anti-tumor effect of the TORC1/2 inhibitor MLN0128/TAK-228 against the rapalogs everolimus and deforolimus in an LK-2 NFE2L2 mutant squamous cell lung cancer xenograft. Decrease in tumor volume was seen only in xenografts treated with MLN0128/TAK-228 (-55% tumor reduction).

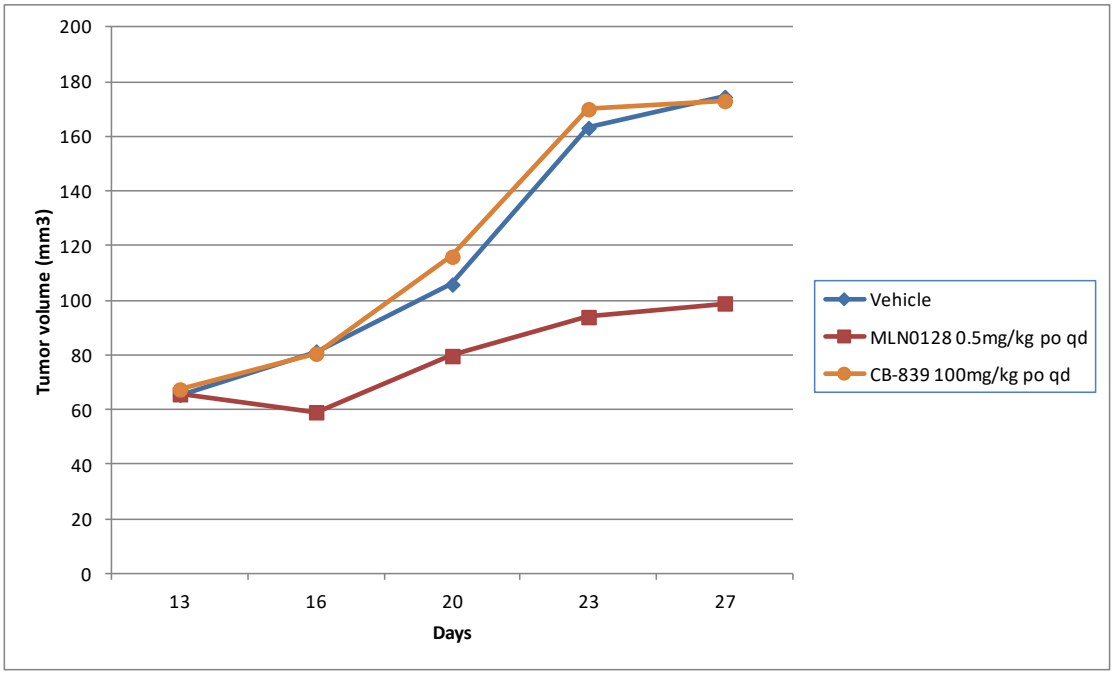



**Figure 10.** Anti-tumor effect of the TORC1/2 inhibitor MLN0128/TAK-228 against an A549 KRAS mutant/KEAP1 null lung cancer xenograft. MLN0128/TAK-228 led to a decrease in tumor volume not seen with the glutaminase inhibitor CB-839.

## 2.5 Correlative Studies Background

### mTOR inhibition

Inhibition of TORC1/2 signaling by MLN0128 (TAK-228) leads to changes in phosphorylation of the TORC1 downstream targets S6 and 4EBP1 (↓pS6 and p4EBP1) and a TORC2 target AKT (↓pAKT) in cell lines of various tumor types and genetic backgrounds, such as HER2-amplified trastuzumab-sensitive and –resistant breast cancer cells (De et al., 2012), breast ductal T47D cell line with PI3KCA mutation (Investigator's Brochure), metastatic RCC (Wang et al., 2011), endometrial cell lines (Kessler et al., 2012), and prostate cancer line PC-3 with PTEN deletion (Investigator's Brochure). Dual TORC1/2 inhibition mitigates the feedback activation of AKT, known to cause resistance to TORC1-selective inhibitors such as rapamycin (De et al., 2012). Its effect in squamous cell lung cancer models with *NFE2L2* and *KEAP1* mutations is shown in Section 2.3, where a decrease in pS6 and p4EBP1 was also seen.

### Reverse phase protein array analysis (RPPA)

There are a number of practical limits associated with pharmacodynamic testing in cancers where the majority of tissue material comes from small needle core biopsies. These include tumor heterogeneity necessitating microdissection, small total protein yields, the need to snap- freeze samples at -80C to preserve phosphoprotein stability, and the demand for a quantitative assay. Immunohistochemistry, which is the most readily available proteomic test, is hampered by its qualitative nature. Western blotting, which can be quantitative depending on the specificity of the antibody used, has poor multiplex capabilities.

This study incorporates reverse phase protein array (RPPA) analysis as an exploratory biomarker analysis to determine the pharmacodynamic effect of MLN0128 (TAK-228) in optional pre- and on-treatment tumor biopsies. RPPA is a highly multiplex quantitative proteomic platform that can test for both phosphorylated and non-phosphorylated proteins. ~~Dr. Lauren Byers at~~ MD Anderson will perform RPPA on these optional paired tumor specimens. A panel of 156 validated antibodies (217 total) is available for testing simultaneously, including key targets for this proposal (p4EBP1, pS6K, AKT pS463 and pT308). We will determine, in descriptive terms, changes in phosphorylation in these specific downstream targets in response to MLN0128 (TAK-228).

Tumor samples will be snap-frozen in liquid nitrogen and stored at -80C. This material will then be shipped frozen to ~~Dr. Byers' laboratory at~~ MD Anderson for analysis.

# 3. PATIENT SELECTION

## 3.1 Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed stage IV or recurrent squamous cell lung cancer or *KRAS* mutant lung cancer that harbors any of the *NFE2L2* mutations or *KEAP1* mutations below. Prioritized *KEAP1* mutations are shown below, but any *KEAP1* mutation will be eligible.

| Eligible and functionally validated Nrf2 amino acids/ mutations (Appendix B) | | | |
|---|---|---|---|
| **NFE2L2 mutation sites** | **Domain** | **Function** | **Validation** |
| aa.17-36 | DLG motif | Keap1 binding site | *in silico and in vitro* |
| aa.76-82 | ETGE motif | Keap1 binding site | *in silico and in vitro* |
| K44, K50, K52, K53, K56, K64, K68 | Lysine ubiquitination targets | Nrf2 degradation | *in silico and in vitro* |
| Exon 2 deletion | DLG/ETGE motifs | Keap1 binding site | *in vitro* |

| Eligible and functionally validated Keap1 amino acids/mutations (Appendix B) | | | |
|---|---|---|---|
| **KEAP1 mutation sites** | **Domain** | **Function** | **Validation** |
| R380, R415, R483 | DGR-CTR | Nrf2 binding residues | *in silico and in vitro* |
| Y525, Q530, N382, Y573, F577, Y334, S363, S602, S555 | DGR-CTR | Nrf2 binding cavity | *in silico and in vitro* |
| G364C, G430C | DGR-CTR | Nrf2 binding cavity | *in silico and in vitro* |
| aa.315-624 | DGR-CTR | Nrf2 binding domain | *in silico* |
| aa.96-100 | BTB HKVVL | Keap1 dimerization | *in silico and in vitro* |
| C151, V155, V167 | BTB | electrophile sensor | *in vitro* |
| D244, S243, C273, C288 | IVR | electrophile sensor | *in vitro* |
| Any frameshift or nonsense mutation | N/A | N/A | N/A |

3.1.2 Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non- nodal lesions and short axis for nodal lesions) as ≥20 mm (≥2 cm) with conventional techniques or as ≥10 mm (≥1 cm) with spiral CT scan, MRI, or calipers by clinical exam. See Section 11 for the evaluation of measurable disease.

3.1.3 Patients must have completed at least 1 prior line of systemic therapy. Patients who have declined first line therapy or for whom first-line therapy would be clinically inappropriate, will be considered eligible for the trial.

3.1.4 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of MLN0128 (TAK-228) in patients <18 years of age and because lung cancer is rare in this age group, children are excluded from this study.

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

3.1.6 Life expectancy of greater than 3 months.

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

- leukocytes ≥3,000/mcL
- absolute neutrophil count ≥1,500/mcL
- platelets ≥100,000/mcL

- total bilirubin — within normal institutional limits
- Fasting Serum Glucose — ≤ 130 mg/dL or HBA1C < 7.0%
- AST(SGOT)/ALT(SGPT) — ≤2.5 × institutional upper limit of normal
- creatinine — within normal institutional limits OR
- creatinine clearance above institutional normal. — ≥50 mL/min/1.73 $m^2$ for patients with elevated creatinine

3.1.8 Patients with controlled Diabetes are allowed on study. Controlled diabetes is defined as FBS ≤ 130 mg/dL in the context of this study

3.1.9 The effects of MLN0128 (TAK-228) on the developing human fetus are unknown. For this reason women of child-bearing potential and men must agree to practice 1 highly effective method of contraception and 1 additional effective (barrier) method, at the same time, prior to study through 90 days (or longer, as mandated by local labeling [eg, USPI, SmPC, etc;]) after the last dose of study drug. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Any woman who becomes pregnant while receiving MLN0128 (TAK-228) will be removed from the trial. Men treated or enrolled on this protocol must also agree to use highly effective barrier contraception prior to the study, for the duration of study participation, and 120 days after completion of MLN0128 (TAK-228) administration. Men must agree not to donate sperm during the course of this study or within 120 days after receiving their last dose of study drug.

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

3.1.11 Ability to swallow oral medications.

3.1.12 Known HIV positive patients who meet the following criteria will be considered eligible:

- CD4 count > 350 cells/$mm^3$
- Undetectable viral load
- Maintained on modern therapeutic regimens utilizing non-CYP-interactive agents

**3.2 Exclusion Criteria**

3.2.1 Patients who have had chemotherapy or radiotherapy within 2 weeks prior to the planned start of study treatment or those who have not recovered to baseline or less than grade 2 from adverse events from prior treatments.

3.2.2 Patients who are receiving any other investigational agents.

3.2.3 Patients with untreated CNS metastases. Patients with treated CNS metastases who are off steroids are eligible.

3.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic

composition to MLN0128 (TAK-228).

3.2.5 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, hypertension, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. No ischemic myocardial or cerebrovascular event, class III or IV heart failure, placement of pacemaker, or pulmonary embolism within six months of receiving first dose of MLN0128 (TAK-228).

3.2.6 Baseline prolongation of the rate-corrected QT interval (QTc) > 480 milliseconds, or history of congenital long QT syndrome, or tosades de pointes.

Pregnant women are excluded from this study because MLN0128 (TAK-228) is an mTOR agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with MLN0128 (TAK-228), breastfeeding should be discontinued if the mother is treated with MLN0128 (TAK-228).

3.2.7 Patients previously treated with an mTOR or PI3K inhibitor.

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

3.2.9 Uncontrolled diabetes mellitus (Fasting plasma glucose > 130mg/dL despite optimal medical management of hyperglycemia)

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

3.2.11 Patients receiving histamine H2 receptor antagonists before enrollment must stop using these medications for at least 24 hours before their first dose of study drug. See section 5.2 for further details on the use of histamine H2 receptor antagonists.

### 3.3 Inclusion of Women and Minorities

NIH policy requires that women and members of minority groups and their subpopulations be included in all NIH-supported biomedical and behavioral research projects involving NIH- defined clinical research unless a clear and compelling rationale and justification establishes to the satisfaction of the funding Institute & Center (IC) Director that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. Exclusion under other circumstances must be designated by the Director, NIH, upon the recommendation of an IC Director based on a compelling rationale and justification. Cost is not an acceptable reason for exclusion except when the study would duplicate data from other sources. Women of childbearing potential should not be routinely excluded from participation in clinical research. Please see http://grants.nih.gov/grants/funding/phs398/phs398.pdf.

# 4. REGISTRATION PROCEDURES

## 4.1 Investigator and Research Associate Registration with CTEP

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

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

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP websites and applications. Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR ***Help Desk*** by email at RCRHelpDesk@nih.gov.

## 4.2 Site Registration

N/A

## 4.3 MSK Patient Registration Process

Confirm in the electronic medical record that the patient has received the Notice of Privacy Practice. This must be obtained before the eligibility confirmation and obtaining of the research informed consent.

Confirm eligibility as defined in the section entitled Patient Selection.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming whether or not the participant is eligible to enroll in the study. Study staff are responsible for ensuring all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Patient Registration).

## 4.4 Informed Consent Procedures

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. Only those individuals designated on the protocol title page as consenting professionals may obtain informed consent. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain

the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

### 4.5 General Guidelines

Eligible patients will be entered on study centrally at Memorial Sloan Kettering Cancer Center by the Study Coordinator.

Following registration, patients should begin protocol treatment within 5-7 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

## 5. TREATMENT PLAN

### 5.1 Agent Administration

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

| **Regimen Description** | | | | | |
|---|---|---|---|---|---|
| ***Agent*** | ***Premedications; Precautions*** | ***Dose*** | ***Route*** | ***Schedule*** | ***Cycle Length*** |
| MLN0128 (TAK-228) | none | 3mg | oral | daily continuous | 28 days (4 weeks) |

Patients will be treated MLN0128 (TAK-228) 3mg oral daily continuously on an every 4 week schedule. MLN0128 (TAK-228) will be taken at the same time every day. The capsules should not be opened, chewed, or crushed. The patient will be given a medication diary at the beginning of each cycle of therapy. The medication diary, along with the pill bottles and any unused drug, will be returned to the clinic staff at the end of each cycle.

In cases where a subject misses dosing at his/her dosing time, the subject may still take the dose within 12 hours of the regular dosing time (subjects should not take 2 consecutive daily doses within 12 hours of each other). Subjects who vomit shortly after receiving MLN0128 (TAK-228) will not

receive a replacement dose. If confirmed that the study drug has been vomited, the dose should be noted as having been missed.

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

## 5.2 General Concomitant Medication and Supportive Care Guidelines

Multiple human metabolizing enzymes are involved in the Phase I metabolism of MLN0128 (TAK-228). When normalized for human liver content, the CYP isoforms CYP3A4, CYP2C9, and CYP2C19 appear to contribute to MLN0128 (TAK-228) metabolism. MLN0128 (TAK-228) displayed low potential (IC50 > 25 μM) for inhibition of the major human CYP isoforms.

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

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

Because there is a potential for interaction of MLN0128 (TAK-228) with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes.

**Management of Hyperglycemia**

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

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

indicated. The investigator should consult an endocrinologist, if needed, to aid in optimizing the patient's hyperglycemia treatment plan.

It is recommended that patients with elevated fasting blood glucose be initially treated with a fast acting insulin sensitizer such as metformin at 500 mg orally QD, and titrate up to a maximum of 1000 mg orally BID as needed. Concurrent addition to metformin of DPP-4 inhibitors (eg, sitagliptin or vildagliptin) and/or insulin should also be considered. Oral sulfonylureas (eg, glipizide or glyburide) should be used with caution, due to the higher risk of inducing hypoglycemia in patients. The dose of oral hypoglycemic agents should be adjusted in patients with renal insufficiency. In addition, patients should be encouraged to follow a low carbohydrate diet once hyperglycemia is first observed.

If any fasting serum glucose reading performed at the site indicates hyperglycemia (>ULN or ≥110 mg/dL), the study staff should first confirm that the patient was fasting at the time of blood specimen collection (ie, nothing by mouth for at least 8 hours before collection).

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

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

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

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

## 5.3 Duration of Therapy

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

- Disease progression (at the discretion of the PI, patients who continue to derive clinical benefit

may be treated beyond progression; see Section 5.3.1 for details),

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

5.3.1 Treatment Beyond Progression

Patients can be treated beyond progression at the discretion of the PI for clinical benefit and will undergo scans per the usual schedule. In these instances, the scan demonstrating RECIST PD will be used as a new baseline to determine further progression. Patients will be removed from the study if RECIST PD is achieved relative to the new baseline scan unless otherwise discussed with the Principal Investigator, if there is intolerable toxicity or if the patient withdraws consent.

### 5.4 Duration of Follow Up

Patients will be followed for 4 weeks after removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

### 5.5 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.4 applies. If a patient is removed for any reason other than disease progression, he or she will be replaced. The reason for study removal and the date the patient was removed must be documented in the Case Report Form. Patients can, at the discretion of the PI, receive treatment beyond progression for clinical benefit (see section 5.3.1 for details).

## 6. DOSING DELAYS/DOSE MODIFICATIONS

Toxicities will be graded using the NCI CTCAE Version 4.0 and dose modifications of the study drugs will be made for hematologic and other toxicities. Patients experiencing study drug related toxicities that require a delay in dosing for >21 days will be discontinued from further participation in this study. In the event that patients must have treatment delayed with a treatment cycle due to toxicities, those doses held during a cycle will not be made up.

| Dose Level | *MLN0128 (TAK-228)* Dose |
|---|---|
| 0 | 3 mg flat dose daily continuous |
| -1 | 2 mg flat dose daily continuous |
| -2 | 2 mg daily 5 days/ week |
| -3 | 1 mg 5 days/week |
| -4 | Withdraw from study |

**Treatment Modification for MLN0128 (TAK-228)-Related Adverse Events**

| Event | CTCAE. V4 Grade | Action to be Taken |
|---|---|---|
| **QTc prolongation** | ≥ Grade 3 or > 60ms change from baseline on at least two separate ECGs | **First Occurrence:**<br>• Omit MLN0128 (TAK-228) dose<br>• Perform an analysis of potassium and magnesium, and if below lower limit of normal, correct with supplements to within normal limits. Concomitant medication usage must be reviewed.<br>• Perform a repeat ECG within one hour of the first QTcF of 500 ms<br>• If QTcF remains > 500 ms, repeat ECG as clinically indicated, but at least once a day until the QTcF returns to < 480 ms. Seek cardiologist input<br>• Once QTcF prolongation has resolved, MLN0128 (TAK-228) may be restarted at a one lower dose level<br><br>**Second Occurrence:**<br>• Permanently discontinue patient from the study treatment |
| **Other Cardiac Events** | Grade 1-2 | Maintain dose level of MLN0128 (TAK-228) |
| | Grade 3 | Omit dose of MLN0128 (TAK-228) until resolved to ≤ Grade 1<br>• If resolved within 7 days, then resume at same dose level of MLN0128 (TAK-228).<br>• If resolved in >7 days, resume at ↓1 dose level of MLN0128 (TAK-228).<br>• If not resolved in 21 days, then discontinue patient from study treatment. |

| | | |
|---|---|---|
| | Grade 4 | Permanently discontinue patient from the study treatment |

**Treatment Modification for MLN0128 (TAK-228)-Related Adverse Events**

| Event | CTCAE. V4 Grade | Action to be Taken |
|---|---|---|
| **ENDOCRINE** | | |
| **Fasting Plasma Glucose (FPG) (2 hrs fasting)** | Grade 1 | Maintain dose level of MLN0128 (TAK-228), check FPG every week<br>• Initiate or intensify with appropriate anti-diabetic treatment as per investigator's discretion<br>• Instruct patient to follow dietary guidelines according to local and/or institutional standards for management of diabetes mellitus (such as those provided by the American Diabetes Association) during the study<br>• Consider use of oral anti-hyperglycemic therapy such as metformin<br>Check FGP daily for 8 weeks, then continue checking at least every 1 week |

| | | |
|---|---|---|
| | Grade 2 | **First Occurrence:**<br>• If signs or symptoms of hyperglycemia (for example, mental status changes, excessive thirst, polyuria), omit dose of MLN0128 (TAK-228) immediately and manage as for Grade 3 hyperglycemia (below)<br>• If asymptomatic, maintain MLN0128 (TAK-228) dose and re-check FPG within 24 hours. If grade worsens or improves then follow specific grade recommendations:<br>o If FPG remains at Grade 2:<br>▪ Maintain dose level of MLN0128 (TAK-228)<br>▪ Initiate or intensify with appropriate anti-diabetic treatment<br>▪ Instruct patient to follow dietary guidelines according to local and/or institutional standards for management of diabetes mellitus (such as those provided by the American Diabetes Association) during the study<br>▪ Consider use of oral anti-hyperglycemic therapy such as metformin<br>o If FPG does not resolve to ≤ Grade 1 within 7 days after initiation/intensifying anti-diabetic treatment:<br>▪ Omit dose of MLN0128 (TAK-228) if |

| Treatment Modification for MLN0128 (TAK-228)-Related Adverse Events | | |
|---|---|---|
| **Event** | **CTCAE. V4 Grade** | **Action to be Taken** |
| | | not already done<br>▪ Monitor FPG at least weekly until FPG resolves to ≤ Grade 1<br>▪ Then re-start MLN0128 (TAK-228) and ↓1 dose level<br>▪ Continue with anti-diabetic treatment<br>▪ Check FPG weekly for 8 weeks, then continue checking every 2 weeks<br><br>**Second and Subsequent Occurrence:**<br>• Maintain dose level of MLN0128 (TAK-228), re- check FPG within 24 hours. If grade worsens or improves then follow specific grade recommendations. If FPG remains at Grade 2:<br>o Omit dose of MLN0128 (TAK-228)<br>o Initiate or intensify with appropriate anti-diabetic treatment<br>o Monitor FPG at least twice weekly until FPG resolves to ≤ Grade 1<br>o Then re-start MLN0128 (TAK-228) and ↓1 dose level<br>o Continue with anti-diabetic treatment<br>o Check FPG at least weekly for 8 weeks, then continue checking at least every 2 weeks |

| | | |
|---|---|---|
| | Grade 3 | Immediately omit MLN0128 (TAK-228), initiate or intensify medication with appropriate anti-diabetic treatment, re-check FPG within 24 hours. If grade worsens or improves then follow specific grade recommendations.<br><br>• If FPG remains at Grade 3:<br>o Administer intravenous hydration and intervention for electrolyte/ketoacidosis/hyperosmolar disturbances as clinically appropriate<br>o Continue to omit dose of MLN0128 (TAK-228)<br>o Monitor FPG at least twice weekly until FPG resolves to ≤ Grade 1<br>o If FPG resolves to ≤ Grade 1 in 7 days or less, then re-start MLN0128 (TAK-228) at the same dose level<br><br>• If FPG remains greater than Grade 1 severity for |

| Treatment Modification for MLN0128 (TAK-228)-Related Adverse Events | | |
|---|---|---|
| **Event** | **CTCAE. V4 Grade** | **Action to be Taken** |
| | | more than 7 days, then discontinue patient from study treatment:<br>o Initiate or continue anti-diabetic treatment as appropriate<br>o Instruct patient to follow dietary guidelines according to local and/or institutional standards for management of diabetes mellitus (such as those provided by the American Diabetes Association) during the study<br>o Consider use of oral anti-hyperglycemic therapy such as metformin<br>o Check FPG at least weekly for 8 weeks, then continue checking at least every 2 weeks<br><br>For non-fasting plasma glucose >250-500mg/dL (> 13.9 – 27.8 mmol/L) accompanied by signs/symptoms of hyperglycemia (for example, mental status changes, excessive thirst, polyuria), or presence of blood or urine ketones, omit MLN0128 (TAK-228) and follow guidance for management of Grade 3 fasting plasma glucose (FPG). |

| | | |
|---|---|---|
| | Grade 4 | Immediately omit dose of MLN0128 (TAK-228), initiate or intensify medication with appropriate anti-diabetic treatment, re-check within 24 hours, if confirmed Grade 4:<br><br>• Administer intravenous hydration and intervention for electrolyte/ketoacidosis/hyperosmolar disturbances as clinically appropriate<br>• Discontinue patient from the study. Instruct patient to follow dietary guidelines according to local and/or institutional standards for management of diabetes mellitus (such as those provided by the American Diabetes Association) during the study<br>• Consider use of oral anti-hyperglycemic therapy such as metformin<br>• Check FPG weekly for 8 weeks, then continue checking at least every 2 weeks if clinically indicated |

| Treatment Modification for MLN0128 (TAK-228)-Related Adverse Events | | |
|---|---|---|
| **Event** | **CTCAE. V4 Grade** | **Action to be Taken** |
| | | For non-fasting plasma glucose >500 mg/dL (> 27.8 mmol/L) accompanied by signs/symptoms of hyperglycemia (for example, mental status changes, excessive thirst, polyuria), or presence of blood or urine ketones, discontinue MLN0128 (TAK-228) and following guidance for management of Grade 4 fasting plasma glucose (FPG). |
| **GASTROINTESTINAL** | | |
| **Mucositis oral** | Grade 1 | Maintain MLN0128 (TAK-228) dose level |
| | Grade 2 | Maintain MLN0128 (TAK-228) dose level if tolerable, if toxicity becomes intolerable omit dose of MLN0128 (TAK-228) until resolved to Grade $\leq$1, then restart at the same dose level |
| | Grade 3 | Omit dose of MLN0128 (TAK-228) until resolved to Grade $\leq$1, then restart at $\downarrow$1 dose level of MLN0128 (TAK-228), If not resolved in 21 days, then discontinue patient from study treatment. |
| | Grade 4 | Permanently discontinue patient from the study treatment |
| **GENERAL DISORDERS** | | |
| **Fatigue (asthenia)** | Grade 1-2 | Maintain MLN0128 (TAK-228) dose level |
| | Grade 3 | Omit dose of MLN0128 (TAK-228) until resolved to $\leq$ Grade 1, then:<br>• If resolved in $\leq$ 7 days, maintain dose level of MLN0128 (TAK-228)<br>• If resolved in > 7 days, $\downarrow$ 1 dose level of MLN0128 (TAK-228)<br>• If not resolved in 21 days, then discontinue patient from study treatment. |
| | Grade 4 | Patients who develop Grade 4 nonhematological toxicities (with the exception of isolated non-clinically significant laboratory values) should permanently discontinue study treatment, unless they derive clinical benefit, in which case they may be retreated at a reduced dose level after recovery to $\leq$ Grade 1 severity. |
| **HEMATOLOGIC** | | |
| **Neutropenia (ANC)** | Grade 1-2 | Maintain dose level of MLN0128 (TAK-228) |

| | | |
|---|---|---|
| | Grade 3 | Omit dose of MLN0128 (TAK-228) until resolved to ≤ Grade 1 or baseline, then:<br>• If resolved in ≤ 7 days, maintain dose level of MLN0128 (TAK-228)<br>• If resolved in > 7 days, resume treatment at ↓1 dose level of MLN0128 (TAK-228)<br>• If not resolved in 21 days, then discontinue |

| Treatment Modification for MLN0128 (TAK-228)-Related Adverse Events | | |
|---|---|---|
| **Event** | **CTCAE. V4 Grade** | **Action to be Taken** |
| | | patient from study treatment. |
| | Grade 4 | Permanently discontinue patient from the study treatment |
| **Febrile Neutropenia** | Grade 3 | Omit dose of MLN0128 (TAK-228) until resolved to Grade 1 or baseline, then:<br>• If resolved in ≤ 7 days, resume treatment at ↓1 dose level of MLN0128 (TAK-228)<br>• If resolved in > 7 days, discontinue MLN0128 (TAK-228) |
| **Thrombocytopenia** | Grade 1-2 | Maintain dose level of MLN0128 (TAK-228) |
| | Grade 3 | Omit dose of MLN0128 (TAK-228) until resolved to ≤ Grade 1 or baseline then:<br>• If resolved in ≤ 7 days, resume treatment at ↓ 1 dose level of MLN0128 (TAK-228)<br>• If resolved in > 7 days, discontinue MLN0128 (TAK-228) |
| | Grade 4 | Permanently discontinue patient from the study treatment |
| **HEPATIC** | | |
| **Bilirubin** | Grade 1 | Maintain dose level of MLN0128 (TAK-228) |
| | Grade 2 (with ALT or AST grade 1 or 2) | Omit dose of MLN0128 (TAK-228) until resolved to ≤ Grade 1 or baseline, then resume at the same dose level of MLN0128 (TAK-228) |
| | Grade 3 (with ALT or AST grade 1 or 2) | Omit dose of MLN0128 (TAK-228) until resolved to ≤ Grade 1 or baseline, then:<br>• If resolved in ≤ 7 days, maintain same dose level of MLN0128 (TAK-228)<br>• If resolved in > 7 days but less than 21 days, resume treatment at ↓ 1 dose level of MLN0128 (TAK-228).<br>• If not resolved in 21 days, then discontinue patient from study treatment. |
| | Grade 4 | Permanently discontinue patient from the study treatment |

| Treatment Modification for MLN0128 (TAK-228)-Related Adverse Events | | |
|---|---|---|
| **Event** | **CTCAE. V4 Grade** | **Action to be Taken** |
| **AST or ALT** | Grade 1 or Grade 2 (without total bilirubin elevation > grade 1) | Maintain dose level of MLN0128 (TAK-228) |
| | Grade 3 (without total bilirubin elevation > grade 1) | Omit dose of MLN0128 (TAK-228) until resolved to ≤ Grade 1 or baseline, then:<br>• If resolved in ≤ 7 days, maintain dose level of MLN0128 (TAK-228)<br>• If resolved in > 7 days, resume treatment at ↓1 dose level of MLN0128 (TAK-228)<br>• If not resolved in 21 days, then discontinue patient from study treatment.<br>• Permanently discontinue study treatment if in combination with Grade 2 total bilirubin elevation when alternative causes cannot be identified (ie, Hy's Law); |
| | Grade 4 | Permanently discontinue study treatment if in combination with Grade 2 total bilirubin elevation when alternative causes cannot be identified (ie, Hy's Law). |
| **RENAL** | | |
| **Creatinine** | Grade 1 | Maintain dose level of MLN0128 (TAK-228) |
| | Grade 2 | Omit dose of MLN0128 (TAK-228) until resolved to ≤ Grade 1 or baseline, then resume treatment at the same dose level. Patients will be instructed to increase their fluid intake until resolution to ≤ Grade 1 or baseline. |
| | Grade 3 | Omit dose of MLN0128 (TAK-228) until resolved to ≤ Grade 1 or baseline then:<br>• If resolved in ≤ 7 days, maintain dose level of MLN0128 (TAK-228)<br>• If resolved in > 7 days, resume treatment at ↓ 1 dose level of MLN0128 (TAK-228)<br>• If not resolved in 21 days, then discontinue patient from study treatment. |
| **RESPIRATORY** | | |
| | Grade 1 | Maintain dose level of MLN0128 (TAK-228) |

| | | |
|---|---|---|
| **Non-infectious Pneumonitis** | Grade 2 | Omit dose of MLN0128 (TAK-228) until resolved to Grade $\leq$ 1, then:<br>• Restart at ↓1 dose level of MLN0128 (TAK-228)<br>• Discontinue MLN0128 (TAK-228) if failure to recover within 4 weeks |
| | Grade 3 | Omit dose of MLN0128 (TAK-228) until resolved to Grade $\leq$ 1, then:<br>• Consider re-initiating MLN0128 (TAK-228) at ↓1 dose level of MLN0128 (TAK-228)<br>• If toxicity recurs at Grade 3, discontinue MLN0128 (TAK-228) |

| Treatment Modification for MLN0128 (TAK-228)-Related Adverse Events | | |
|---|---|---|
| **Event** | **CTCAE. V4 Grade** | **Action to be Taken** |
| | Grade 4 | Permanently discontinue patient from the study treatment |
| **SKIN AND SUBCUTANEOUS TISSUE DISORDERS** | | |
| **Rash (maculo-papular)** | Grade 1 | Maintain dose level of MLN0128 (TAK-228). Consider initiation appropriate skin toxicity therapy (such as antihistamines, topical corticosteroids) |
| | Grade 2 | Maintain dose level of MLN0128 (TAK-228). Initiate/intensify appropriate skin toxicity therapy (such as antihistamines, topical corticosteroids) |
| | Grade 3 | Omit dose of MLN0128 (TAK-228) until resolved to Grade ≤ 1, then:<br>• If resolved in ≤ 7 days, maintain at same dose of MLN 0218<br>• If resolved in > 7 days, resume at ↓1 dose level of MLN0128 (TAK-228)<br>• If not resolved in 21 days, then discontinue patient from study treatment. |
| | Grade 4 | Patients who develop Grade 4 rash should permanently discontinue study treatment, unless they derive clinical benefit, in which case they may be retreated at a reduced dose level after recover to ≤ Grade 1 severity. Grade 4 rash is defined as rash acneifom/ papulopustular with papules and/or pustules covering any % body surface area, which may or may not be associated with symptoms of pruritus or tenderness, and are associated with extensive superinfection with intravenous (IV) antibiotics indicated; life threatening consequences (NCI CTCAE Version 4.03, effective date 14 June 2010). |
| **INVESTIGATIONS** | | |
| **Cholesterol high and/or hypertriglyceridemia** | Grade 1 | Maintain MLN0128 (TAK-228) dose level |
| | Grade 2 | Maintain dose if tolerable, if toxicity becomes intolerable, omit dose MLN0128 (TAK-228) until resolved to ≤ 1, then restart MLN0128 (TAK-228) as the same dose level |
| | Grade 3 | Omit dose of MLN0128 (TAK-228) until resolved to Grade ≤ 1, then restart at ↓1 dose level of MLN0128 (TAK-228) |
| | Grade 4 | Permanently discontinue patient from the study treatment |

| OTHER ADVERSE EVENTS | | |
|---|---|---|
| **Other AEs** | Grade 1-2 | Maintain MLN0128 (TAK-228) |
| | Grade 3 | Omit dose of MLN0128 (TAK-228) until resolved to ≤Grade 1, then:<br>• If resolved in ≤ 7 days, maintain at same dose of MLN 0218<br>• If resolved in > 7 days, resume at ↓1 dose level of MLN0128 (TAK-228)<br>• If not resolved in 21 days, then discontinue patient from study treatment. |
| | Grade 4 | Permanently discontinue patient from the study treatment |
| | | **Note:** Omit dose for ≥ Grade 3 vomiting or Grade 3 nausea only if the vomiting or nausea |

## 7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

### 7.1 Comprehensive Adverse Events and Potential Risks List (CAEPR) For MLN0128 (TAK-228, NSC 768435)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 390 patients.* Below is the CAEPR for MLN0128 (TAK-228).

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

### 7.1.1 CAEPR for MLN0128 (TAK-228)

**Version 2.3, July 28, 2019**[1]

| **Adverse Events with Possible Relationship to MLN0128 (TAK-228) (CTCAE 5.0 Term) [n= 390]** | | | **Specific Protocol Exceptions to Expedited Reporting (SPEER)** |
|---|---|---|---|
| **Likely (>20%)** | **Less Likely (<=20%)** | **Rare but Serious (<3%)** | |
| BLOOD AND LYMPHATIC SYSTEM DISORDERS | | | |
| | Anemia | | ***Anemia (Gr 2)*** |
| CARDIAC DISORDERS | | | |
| | | Cardiac arrest | |
| | | Ventricular fibrillation | |
| GASTROINTESTINAL DISORDERS | | | |
| | Abdominal pain | | ***Abdominal pain (Gr 2)*** |
| Constipation | | | ***Constipation (Gr 2)*** |
| Diarrhea | | | ***Diarrhea (Gr 2)*** |
| | Dry mouth | | ***Dry mouth (Gr 2)*** |
| | Dyspepsia | | |
| Mucositis oral | | | ***Mucositis oral (Gr 2)*** |
| Nausea | | | ***Nausea (Gr 3)*** |
| Vomiting | | | ***Vomiting (Gr 3)*** |
| GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS | | | |
| | Edema limbs | | |
| Fatigue | | | ***Fatigue (Gr 3)*** |
| | Fever | | ***Fever (Gr 2)*** |
| | General disorders and administration site conditions - Other (mucosal inflammation) | | ***General disorders and administration site conditions - Other (mucosal inflammation) (Gr 2)*** |
| INFECTIONS AND INFESTATIONS | | | |
| | Urinary tract infection | | ***Urinary tract infection (Gr 2)*** |
| INVESTIGATIONS | | | |
| | Creatinine increased | | ***Creatinine increased (Gr 2)*** |
| | | Electrocardiogram QT corrected interval prolonged | |
| | Platelet count decreased | | ***Platelet count decreased (Gr 2)*** |
| | Weight loss | | ***Weight loss (Gr 2)*** |
| METABOLISM AND NUTRITION DISORDERS | | | |
| Anorexia | | | ***Anorexia (Gr 2)*** |
| | Dehydration | | ***Dehydration (Gr 2)*** |
| Hyperglycemia | | | ***Hyperglycemia (Gr 3)*** |
| | Hypokalemia | | ***Hypokalemia (Gr 2)*** |
| | Hypomagnesemia | | ***Hypomagnesemia (Gr 2)*** |
| | Hypophosphatemia | | ***Hypophosphatemia (Gr 2)*** |
| MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS | | | |
| | Arthralgia | | |
| | Back pain | | ***Back pain (Gr 2)*** |

| Adverse Events with Possible Relationship to MLN0128 (TAK-228) (CTCAE 5.0 Term) [n= 390] | | | Specific Protocol Exceptions to Expedited Reporting (SPEER) |
|---|---|---|---|
| **Likely (>20%)** | **Less Likely (<=20%)** | **Rare but Serious (<3%)** | |
| | Pain in extremity | | |
| NERVOUS SYSTEM DISORDERS | | | |
| | Dizziness | | ***Dizziness (Gr 2)*** |
| | Dysgeusia | | ***Dysgeusia (Gr 2)*** |
| | Headache | | ***Headache (Gr 2)*** |
| PSYCHIATRIC DISORDERS | | | |
| | Insomnia | | |
| RENAL AND URINARY DISORDERS | | | |
| | Acute kidney injury | | |
| RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS | | | |
| | Cough | | ***Cough (Gr 2)*** |
| | Dyspnea | | ***Dyspnea (Gr 2)*** |
| | Oropharyngeal pain | | |
| | | Pneumonitis | |
| SKIN AND SUBCUTANEOUS TISSUE DISORDERS | | | |
| Pruritus | | | ***Pruritus (Gr 2)*** |
| Rash maculo-papular | | | ***Rash maculo-papular (Gr 2)*** |

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

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

**BLOOD AND LYMPHATIC SYSTEM DISORDERS** - Blood and lymphatic system disorders - Other (hyperviscosity syndrome); Blood and lymphatic system disorders - Other (Raynaud's phenomenon); Febrile neutropenia
**CARDIAC DISORDERS** - Heart failure; Pericardial effusion; Sinus tachycardia; Ventricular arrhythmia
**EYE DISORDERS** - Blurred vision; Eye pain; Photophobia; Vision decreased
**GASTROINTESTINAL DISORDERS** - Abdominal distension; Colitis; Dysphagia; Esophagitis; Gastritis; Gastroesophageal reflux disease; Gastrointestinal disorders - Other (intestinal obstruction); Gastrointestinal disorders - Other (intestinal perforation); Gastrointestinal disorders - Other (salivary hypersecretion); Hemorrhoids; Ileus; Oral pain; Pancreatitis; Small intestinal obstruction; Small intestinal perforation; Toothache
**GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS** - Chills; Flu like symptoms; Gait disturbance; General disorders and administration site conditions - Other (groin pain); Malaise; Non-cardiac chest pain; Pain
**HEPATOBILIARY DISORDERS** - Gallbladder obstruction
**IMMUNE SYSTEM DISORDERS** - Allergic reaction
**INFECTIONS AND INFESTATIONS** - Abdominal infection; Infections and infestations - Other (cystitis); Infections and infestations - Other (lower respiratory tract infection); Infections and infestations - Other (mucosal infection); Infections and infestations - Other (parotid gland); Kidney infection; Lung infection; Papulopustular rash; Sepsis; Skin infection; Upper respiratory infection
**INJURY, POISONING AND PROCEDURAL COMPLICATIONS** - Fall; Fracture; Injury, poisoning and

procedural complications - Other (accidental overdose); Injury, poisoning and procedural complications - Other (postoperative fever); Injury, poisoning and procedural complications - Other (subdural hemorrhage); Tracheal obstruction
**INVESTIGATIONS** - Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Blood lactate dehydrogenase increased; Cholesterol high; GGT increased; Lipase increased; Lymphocyte count decreased; Neutrophil count decreased; White blood cell decreased
**METABOLISM AND NUTRITION DISORDERS** - Acidosis; Hypercalcemia; Hyperkalemia; Hypernatremia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hyponatremia; Metabolism and nutrition disorders - Other (severe chronic malnutrition); Metabolism and nutrition disorders - Other (vitamin D deficiency)
**MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS** - Bone pain; Chest wall pain; Flank pain; Generalized muscle weakness; Muscle cramp; Myalgia
**NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)** - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (non-hodgkin lymphoma); Treatment related secondary malignancy
**NERVOUS SYSTEM DISORDERS** - Ataxia; Intracranial hemorrhage; Lethargy; Nervous system disorders - Other (carotid artery occlusion); Nervous system disorders - Other (neuropathy peripheral); Paresthesia; Radiculitis; Stroke; Tremor
**PSYCHIATRIC DISORDERS** - Anxiety; Confusion; Depression; Psychiatric disorders - Other (mental status changes)
**RENAL AND URINARY DISORDERS** - Dysuria; Hematuria; Proteinuria; Renal and urinary disorders - Other (strangury)
**REPRODUCTIVE SYSTEM AND BREAST DISORDERS** - Vaginal hemorrhage
**RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS** - Bronchopulmonary hemorrhage; Epistaxis; Hiccups; Hypoxia; Nasal congestion; Pleural effusion; Pleuritic pain; Pneumothorax; Postnasal drip; Productive cough
**SKIN AND SUBCUTANEOUS TISSUE DISORDERS** - Alopecia; Dry skin; Hyperhidrosis; Rash acneiform; Urticaria
**VASCULAR DISORDERS** - Flushing; Hypertension; Hypotension; Thromboembolic event

**Note**: MLN0128 (TAK-228) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

## 7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting beginning April 1, 2018 (investigators may continue to collect and locally store AE data in CTCAE v4.0). All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**

  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the

SPEER column, Section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.

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

## 7.3 Expedited Adverse Event Reporting

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

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

7.3.2 Distribution of Adverse Event Reports

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

7.3.3 Expedited Reporting Guidelines

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

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

Death due to progressive disease should be reported as **Grade 5 "Disease Progression"** in the system organ class (SOC) "General disorders and administration site conditions." Evidence that the death was a manifestation of underlying disease

(*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

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

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

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

**ALL SERIOUS** adverse events that meet the above criteria MUST be immediately reported to the NCI via electronic submission within the timeframes detailed in the table below.

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

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

**Expedited AE reporting timelines are defined as:**

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

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

- All Grade 3, 4, and Grade 5 AEs

**Expedited 10 calendar day reports for:**

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

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

Effective Date: May 5, 2011

## 7.4 Routine Adverse Event Reporting

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

SAEs requiring submission to the HRPP Office must be reported within 5 calendar days of learning of the event. SAEs not requiring submission to the HRPP Office must be saved in the regulatory binder within 30 calendar days of learning of the event. The report should contain the following information:

The following fields in this section will pre-populate based on the protocol information entered in PIMS.

- IRB Number
- Protocol Title
- Protocol Status
- Sponsoring Department
- Principal Investigator
- Protocol Type
- Protocol Category
- Sponsor

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - An explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

## 7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary

malignancy is not considered a metastasis of the initial neoplasm.

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

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

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

### 7.6 Second Malignancy

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

## 8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agent administered in this study can be found in Section 7.1.

### 8.1 MLN0128 (TAK-228)

#### 8.1.1 MLN0128 (TAK-228) Drug Information and Availability

**Other Names:** TAK-228, INK128

**Classification:** mTOR inhibitor, TORC1/2

**CAS Registry Number:** 1224844

**Molecular Formula:** $C_{15}H_{15}N_7O$ **M.W.:** 309.3

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

**Mode of Action:** MLN0128 is a non-rapamycin analog mTOR (mechanistic target of

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

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

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

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

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

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

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

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

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

8.1.2 MLN0128 (TAK-228) Ordering and Accountability

8.1.2.1 NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between

institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

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

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

# 9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

## 9.1 Integral Laboratory or Imaging Studies

### 9.1.1 *NFE2L2, KEAP1 and KRAS mutation assessment*

A detailed explanation of the rationale for targeting *NFE2L2* and *KEAP1* mutations in squamous cell lung cancers and KRAS mutant lung cancers harboring NFE2L2 or KEAP1 alterations with MLN0128 (TAK-228) can be found in Section 2.0. Briefly, determination of mutation status will be performed through prospective New York State Department of Health approved targeted exon sequencing of patient tumor samples in a CLIA laboratory (IMPACT testing). Results are posted in the patient EMR and discussed in detail by the ordering oncologist. About 70 patients will need to be tested to identify 10 *NFE2L2* mutations. About 140 will need to be tested to identify 10 *KEAP1* mutations, given the restriction in the types of *KEAP1* mutations that are eligible. As of February 2014, 64 patients, predominantly with stage IV disease, have been tested in the past year. We have identified 15 cases with NFE2L2 mutations and 10 with KEAP1 mutations. These numbers are in keeping with our anticipated accrual rates for the

protocol.

IMPACT testing will be performed under MSKCC's clinical consent process and *NFE2L2, KEAP1,* and *KRAS* mutation results obtained from the medical records.

9.1.1.1 Collection of Specimen(s)

NA

9.1.1.2 Handling of Specimens(s)

NA

9.1.1.3 Shipping of Specimen(s)

N/A

9.1.1.4 Site(s) Performing Correlative Study

Memorial Sloan Kettering Cancer Center

## 9.2 Exploratory/Ancillary Correlative Studies

9.2.1 *Pharmacodynamic effect of MLN0128 (TAK-228) through reverse phase proteomic analysis*

There are a number of practical limits associated with pharmacodynamic assessment of targeted therapies in cancers where the majority of tissue material comes from small needle core biopsies. These include tumor heterogeneity, small total protein yields, a common requirement to snap-freeze samples at -80C to preserve phosphoprotein stability, and the paucity of quantitative assays to assess signaling changes. Immunohistochemistry, which is the most readily available proteomic test, is hampered by its qualitative nature. Western blotting, which can be quantitative depending on the specificity of the antibody used, has poor multiplex capabilities.

The pre-clinical rationale for assessing dynamic changes in mTOR signaling is detailed in Section 2.3. This correlative study incorporates reverse phase protein array (RPPA) analysis as an exploratory biomarker test to determine the pharmacodynamic effect of MLN0128 (TAK-228) in optional pre- and on-treatment tumor biopsies. RPPA is a highly multiplex quantitative proteomic platform that can test for both phosphorylated and non-phosphorylated proteins. Dr. Lauren Byers at MD Anderson will perform RPPA on these optional paired tumor specimens. A panel of 156 validated antibodies (217 total) is available for testing simultaneously, including key targets for this proposal (p4EBP1, pS6K, AKT pS463 and pT308). The endpoint of this study is feasibility, as it is uncertain as to whether the paired small needle biopsies will yield sufficient material, after microdissection, for analysis. We will determine, in an exploratory fashion, what changes in phosphorylation in these specific downstream targets occurs in response to MLN0128

(TAK-228), including percent changes in specific signaling molecules (pS6K, p4EBP1, pAKT as examples) and a qualitative assessment of pathway changes through the generation of heatmaps.

9.2.1.1 Collection of Specimen(s)

Patients will be asked, at the time of initial informed consent to the study, to participate in this optional correlative study which will obtain a biopsy of their tumor before drug dosing and during week 2 (C1D8 +/- 7 days). Patients will have their biopsies snap-frozen in liquid nitrogen and stored in a -80C freezer. A total of up to 4 18-20 gauge needle cores will be obtained, depending on the feasibility as assessed by the physician performing the biopsy. For resection specimens obtained through surgery, a minimum of 0.5 $mm^3$ of tissue will be obtained.

9.2.1.2 Handling of Specimens(s)

Specimens will be obtained through interventional radiology or surgery. The biopsy will be picked up by the research study assistant and placed immediately in a cryotube and stored in liquid nitrogen.

9.2.1.3 Shipping of Specimen(s)

Specimens will be shipped in dry ice shippers to ~~Dr. Lauren Byers at~~ MD Anderson Cancer Center at 1515 Holcombe Boulevard, Houston, TX 77030. ~~Phone: 713-792-6363, email: byersl@mdanderson.org.~~

Samples will be scheduled to arrive Monday-Friday between 9AM-4PM. ~~Dr. Byer's~~ laboratory will be notified in advance to ensure staff is available for receipt. A sample list in Excel format will be included in the dry ice shipper which lists each sample contained in the box. No PHI will be included in the Excel file; only deidentified codes will be provided. Samples will be shipped in batches at the end of study.

9.2.1.4 Site(s) Performing Correlative Study

MD Anderson Cancer Center.

## 10. STUDY CALENDAR

***Schedules shown in the Study Calendar below are provided as an example and should be modified as appropriate.***

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Informed consent is to be obtained within 45 days prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy. Protocol visits, assessments, including radiology assessments must be completed within +/- 7 days. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

| | Pre-Study | Wk 1 | Wk 2 | Wk 3 | Wk 4 | Wk 5 | Wk 6 | Wk 7 | Wk 8 | Wk 9 | Wk 10 | Wk 11 | Wk 12+ | Off Study[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MLN0128 (TAK-228) | | A | | | | A | | | | A | | | | |
| Informed consent | X | | | | | | | | | | | | | |
| Demographics | X | | | | | | | | | | | | | |
| Medical history | X | | | | | | | | | | | | | |
| Concurrent meds | X | X---------- | | | | | | | | | | | X | |
| Physical exam | X | X | | X | | X | | | | X | | | | X |
| Vital signs | X | X | | X | | X | | | | X | | | | X |
| Height | X | | | | | | | | | | | | | |
| Weight | X | X | | | | X | | | | X | | | | X |
| Performance status | X | X | | | | X | | | | X | | | | X |
| CBC w/diff, plts | X | X | | X | | X | | | | X | | | | X |
| Chemistry[a] | X | X | | X | | X | | | | X | | | | X |
| EKG | X | | | | | X | | | | | | | | |
| Adverse event evaluation | | X---------- | | | | | | | | | | | X | X |
| Tumor measurements | X | Tumor measurements are repeated every 8 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease. | | | | | | | | | | | | X |
| Radiologic evaluation | X | Radiologic measurements should be performed every 8 weeks. | | | | | | | | | | | | X |
| B-HCG | X[b] | | | | | | | | | | | | | |
| Optional tumor biopsy | X | | X | | | | | | | | | | | |

A: MLN0128 (TAK-228): Dose as assigned
a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, fasting glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium, fasting lipid panel, amylase, lipase
b: Serum pregnancy test (women of childbearing potential).
c: Off-study evaluation.
d: EKGs will be done at screening, pre-dose at week 5 and as needed after week 5

## 11. MEASUREMENT OF EFFECT

### 11.1 Antitumor Effect – Solid Tumors

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

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

#### 11.1.1 Definitions

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

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

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

#### 11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) 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 (≥1.5 cm) in short axis when assessed by CT scan (CT scan

slice thickness recommended to be no greater than 5 mm [0.5 cm]). 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 [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1 to <1.5 cm] short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

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

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

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

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

### 11.1.3 Methods for Evaluation of Measurable Disease

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

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

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm (≥1 cm) diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

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

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

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their

entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

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

Tumor markers Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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

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

### 11.1.4 Response Criteria

#### 11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (<1 cm).

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 (0.5 cm).

(Note: the appearance of one or more new lesions is also considered progressions).

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

11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm [<1 cm] short axis).

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

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

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

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

11.1.4.3 Evaluation of Best Overall Response

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

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

| Target Lesions | Non-Target Lesions | New Lesions | Overall Response | Best Overall Response when Confirmation is Required* |
|---|---|---|---|---|
| CR | CR | No | CR | ≥4 wks. Confirmation** |
| CR | Non-CR/Non-PD | No | PR | |
| CR | Not evaluated | No | PR | |

| | | | | |
|---|---|---|---|---|
| PR | Non-CR/Non-PD/not evaluated | No | PR | ≥4 wks. Confirmation** |
| SD | Non-CR/Non-PD/not evaluated | No | SD | Documented at least once ≥4 wks. from baseline** |
| PD | Any | Yes or No | PD | no prior SD, PR or CR |
| Any | PD*** | Yes or No | PD | |
| Any | Any | Yes | PD | |

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

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

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

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

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

11.1.5 Duration of Response

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

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

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

11.1.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

## 12. DATA REPORTING / REGULATORY REQUIREMENTS

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

### 12.1 Data Reporting

12.1.1 Method

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis, either by FTP burst of data or via the CDS web application. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site (http://ctep.cancer.gov/reporting/cdus.html).

**Note**: If your study has been assigned to CDUS-Complete reporting, **all** adverse events (both routine and expedited) that have occurred on the study and meet the mandatory CDUS reporting guidelines must be reported via the monitoring method identified above. If your study has been assigned to CDUS-Abbreviated reporting, no adverse event reporting (routine or expedited) is required to be reported via CDUS.

12.1.2 Responsibility for Data Submission

N/A

### 12.2 CTEP Multicenter Guidelines

N/A

### 12.3 Collaborative Agreements Language

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

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can

Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

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

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

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

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

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

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

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

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the

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

Email: ncicteppubs@mail.nih.gov

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

## 13. STATISTICAL CONSIDERATIONS

### 13.1 Study Design/Endpoints

Patient accrual is nearly complete for the three study cohorts: 1) squamous cell lung cancer with *NFE2L2* mutations; 2) squamous cell lung cancer with *KEAP1* mutations; 3) *KRAS* mutant lung cancer with concomitant *NFE2L2* or *KEAP1* aberration. The primary endpoint is objective response and the observed response rates in the three cohorts are: (1) 2/9, (2) 0/5, and (3) 0/5. We propose to amend the protocol to accrue an additional 10 patients for cohorts 1 and 2 to obtain preliminary information on progression free survival and greater precision for the observed response rate. Although no responses were observed in the first 5 patients in cohort 2, the genomic heterogeneity of KEAP1 mutations in squamous cell lung cancer would indicate that further exploration is needed. Cohort 3 will be closed to accrual. The original study design is provided below.

This study utilizes a Simon two-stage design applied to each of the mutation cohorts. The primary endpoint of the study is objective response rate. The null and desired response rates are 5% and 40%. A desired response rate of 40% was selected based on past successful targeted therapy trials in lung cancer. Response rates in these biomarker-led studies are universally equal to or greater than first-line chemotherapy response rates of between 30-40%. In stage 1 of each mutation cohort, 5 patients will enter the study; if there are no responses, the study will be terminated early. If 1 or more patients respond, enrollment will be extended to 10 patients in total. At the end of stage 2, if 2 or more patients responded in either cohort, the trial will be declared effective and worthy of further testing for the respective cohort. This two-stage design yields 90% power to detect the difference at a 10% type I error rate.

Overall response rate (CR+PR) will be calculated separately for each cohort, including exact

95% confidence intervals. Duration of overall response and duration of stable disease as described in Section 11.1.5 will be calculated and summarized.

Secondary endpoints include PFS and the RPPA correlative biomarker analysis. PFS is defined from the start of treatment until progression or death, whichever occurs first. Patients will be censored at the time of the last on-study evaluation if they do not experience the event of interest. Median progression-free survival will be estimated in each cohort from start of treatment using the Kaplan-Meier method with a two-sided 95% confidence interval. The endpoint of the RPPA analysis is feasibility, defined as the ability to procure sufficient quantity and quality of tumor protein for testing. The proportion of patients with sufficient quantity and quality will be calculated, including an exact 95% confidence interval. Single target signaling changes (decrease in pS6K, p4EBP-1 as examples) will be reported as percentages relative to the baseline pre-treatment tumor sample. Larger scale pathway changes will qualitatively represented through heatmaps.

## 13.2 Sample Size/Accrual Rate

Between 5-10 patients were initially accrued in each mutation cohort arm for a total of 20 patients. An additional 10 patients in cohorts 1 and 2 will be added for the extension phase of this study. Accrual rate is estimated at 1 patient in each arm every 2 months.

**PLANNED ENROLLMENT REPORT**

| Racial Categories | Ethnic Categories | | | | Total |
|---|---|---|---|---|---|
| | Not Hispanic or Latino | | Hispanic or Latino | | |
| | Female | Male | Female | Male | |
| American Indian/ Alaska Native | 0 | 0 | 0 | 0 | **0** |
| Asian | 3 | 3 | 0 | 0 | **6** |
| Native Hawaiian or Other Pacific Islander | 0 | 0 | 0 | 0 | **0** |
| Black or African American | 3 | 3 | 0 | 0 | **6** |
| White | 12 | 12 | 2 | 2 | **28** |
| More Than One Race | 0 | 0 | 0 | 0 | **0** |
| **Total** | **18** | **18** | **2** | **2** | **40** |

## 13.3 Reporting and Exclusions

13.3.1 Evaluation of Toxicity

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

13.3.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

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

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

# 14. PROTECTION OF HUMAN SUBJECTS

Prior to enrollment of each patient, the risks, benefits, and objectives of the study will be reviewed with the participant, including a discussion of the possible toxicities, side effects, and alternative, non-protocol treatment options. The patients will be reminded that participation in this clinical trial is voluntary and the patient may withdraw consent at any time.

Risks: The standard second-line chemotherapy for patients with metastatic/recurrent squamous cell lung cancers is docetaxel. There is a chance that this regimen is less effective than docetaxel. Treatment with MLN0128 (TAK-228) will not absolutely preclude later treatment with docetaxel however.

Benefits: It is hoped that this regimen will prove to be more effective than docetaxel historically. If so, this would add an additional treatment option for patients with stage IV/recurrent squamous

cell lung cancers.

Consent process: Participation in this trial is voluntary. All patients will be required to sign a statement of informed consent which must conform to MSK IRB guidelines.

Costs: Patients will be charged for physician visits, routine laboratory and radiologic studies required for monitoring their condition. Patients will not be billed for the study drug or for the optional biopsies, or for testing performed on the optional biopsy specimens.

Alternatives: Alternative treatment options include standard chemotherapy, best supportive care, or participation in other investigational studies.

Confidentiality: Every effort will be made to maintain patient confidentiality. Research and hospital records are confidential. Patient's names or any other personally identifying information will not be used in reports or publications resulting from this study. Qualified monitors from MSK, the NCI, and the FDA may review patient records as required.

Patient safety: Patients are monitored by physicians and oncology nurses who are very familiar with clinical trials. In the case of an adverse reaction, immediate medical attention is available. In the evenings and weekends, we have a 24 hour urgent care facility for outpatients. The PI or co-PIs will also be available at all times to organize any necessary intervention.

Monitoring of data to ensure safety: this study is to be monitored by institutional IRB. This incorporates an independent data and safety monitoring committee established by arrangement with the National Cancer Institute. The analysis of safety will include all patients. Adverse events, including all toxic effects of treatment, will be tabulated individually and summarized by severity and causality.

## 14.1 Privacy

It is the responsibility of the Research Staff to ensure that protocol subjects received the Center's Notice of Privacy Practices. If the subject has not received one, MSK personnel must provide a Notice of Privacy Practices and obtain acknowledgment before the subject participates in the study.

MSK's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

The consent indicates that individualized de identified information collected for the purposes of this study may be shared with other qualified researchers. Only researchers who have received approval from MSK will be allowed to access this information which will not include protected health information, such as the participant's name, except for dates. It is also stated in the Research Authorization that their research data may be shared with other qualified researchers.

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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 ELIGIBLE *NFE2L2, KEAP1* AND *KRAS* MUTATIONS

The below Appendix B provides support for the inclusion of the *NFE2L2, KEAP1* and *KRAS* mutations in this study.

### Functional *NFE2L2* and *KEAP1* domains

Nrf2, the transcription factor encoded by *NFE2L2*, is regulated primarily by degradation through a Keap1-dependent ubiquitin-proteasome pathway. Analysis of the Nrf2 protein more than a decade ago identified 6 highly conserved Neh (Nrf2-ECH homology) domains thought to be functionally important (**Figure 1**). Of these 6 domains, the N-terminal Neh2 domain (aa.1-98) was identified as the canonical Keap1 binding site.(1) Subsequent work showed that within the Neh2 domain, two evolutionarily conserved motifs were present- the DLG motif (aa.17-36) residing in the N-terminal region and the ETGE motif (aa.76-82) residing in the C-terminal portion of the domain. Seven lysine residues N-terminal of the ETGE motif at aa.44, 50, 52, 53, 56, 64, and 68 have also been shown to be necessary for Keap1-dependent ubiquitination and degradation of Nrf2.(2) The importance of the DLG and ETGE motifs is presented in the next section.

Keap1 contains a number of conserved domains the most important of which are Bric-a-Brac (BTB, aa.61-179), the intervening region (IVR, aa.179-315), a double glycine repeat or Kelch repeat (DGR, aa.315-598), and the C-terminal region (CTR, aa.598-624) (**Figure 1**). The BTB domain is thought to assist in dimerization of Keap1. The C-terminal DGR-CTR domain (aa.315-624) forms a six-bladed β-propeller structure and is the key Nrf2 binding domain. What follows is a summary of the functional data available for each domain.

#### *Keap1 DGR-CTR domain and the Nrf2 Neh2 domain are the critical Keap1/Nrf2 binding sites*

The physical interaction between the DGR-CTR domain in Keap1 and the Neh2 domain in Nrf2 is essential in suppressing Nrf2 activity. Padmanabhan et al. performed a structural analysis of the DGR-CTR domains of mouse Keap1 and the DGR-CTR complex with Nrf2.(3) Functionally, mutations in the CTR domain were found to impair Keap1-mediated repression of Nrf2 transcriptional activity. Mutations in this region also altered localization of Nrf2, leading to increased nuclear accumulation consistent with loss of function of Keap1.

A separate structural analysis of the DGR-CTR domain from Keap1 complexed with the Neh2 domain from Nrf2 confirmed that the binding domains within Neh2 resided in the DLG and ETGE motifs.(4) First, a probe of the binding stoichiometry, binding constant, enthalpy change, and entropy change between Neh2 and DGR-CTR showed a biphasic isotherm curve consistent with a two-site binding model. Padmanabhan et al. demonstrated that a β hairpin comprised of aa.77-82 (corresponding to the C-terminal ETGE motif) is the high-affinity binding site for Keap1, and that it binds to the bottom of the β propeller formed

by DGR-CTR (3). Within Keap1, Arg-380, Arg-415, and Arg-483 appear to bind specifically to the two acidic glutamates in the ETGE motif.(4) Consistent with this, mutations in R415 substituting lysine for arginine and R483 substituting glycine for arginine decreased Neh2 binding affinity by an order of magnitude(3). Other amino acids in the DGR-CTR domain cavity exposed to the ETGE β hairpin include Tyr-525 and Gln-530, Asn-382, Tyr-572, Phe-577, Tyr-334, Ser-363, Ser-602, and Ser-555.

Tong et al. also found that a weaker binding site is present between aa.1-30 which contains the DLG motif. A peptide containing the DLG motif spanning aa.17-36 elicited a spectral change in the 3 arginine residues in the DGR-CTR domain of Keap1, similar to what was observed with the ETGE peptide motif. This region also appears to assist in positioning the intervening lysine residues for efficient ubiquitination(5).

In terms of *KEAP1* mutations found in lung cancer, Padmanabhan identified a G364C and G430C mutation in a lung cancer patient sample and cell line, respectively. Both of these mutant proteins impaired the ability of Keap1 to repress Nrf2 activity, co-immunoprecipitate with Nrf2, and sequester Nr2 in the cytoplasm relative to WT Keap1. Modeling these mutant forms of Keap1 within the murine DRG-CTR/Nrf2 structural model showed that G364C abolishes Keap1-Nrf2 interaction by altering the conformation of Ser-363. The hydrophobic nature of cysteine was theorized to be unable to fit within the charged vicinity formed by the key arginine and serine residues in the DGR-CTR cavity. Binding was also abolished with the G430C mutation, presumably by a conformational change that prevents interaction with Glu-79 on Nrf2.

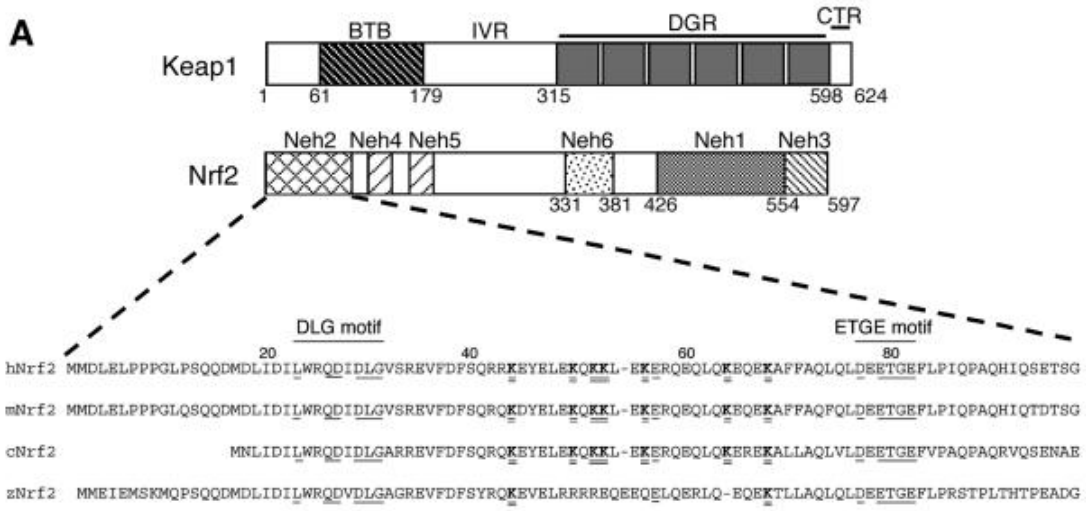


**Figure 1.** Key functional domains for Keap1 and Nrf2. The N-terminal Neh2 domain is the binding site for the negative regulator Keap1. This interaction occurs through the Keap1 C-terminal DGR-CTR domain and both N-terminal (DLG-motif), C-terminal (ETGE motif), and intervening domain (lysine residues, in bold).(4)

### *Keap1 BTB and IVR domains*

The Keap1 BTB domain spanning aa.61-179 is required for homodimerization of Keap1 and for mediating the interaction between Keap1 and Cul3 (forming the E3-ubiquitin ligase complex that degrades Nrf2).(6) Homodimerization appears to be necessary for Keap1 to repress Nrf2 function and to promote Nrf2 ubiquitination.(7) Substitution of the HKVVL sequence (aa.96-100) in the BTB domain with 5 alanine residues abrogated dimerization and did not rescue mice from juvenile mortality when crossed with a *Keap1-/-* background, suggesting that BTB mutations have a dominant negative effect. A separate group found similar results, with a dominant negative BTB construct inhibiting the ability of Keap1 to retain Nrf2 in the cytoplasm.(8)

Lastly, the IVR domain contains 2 cysteine residues and the BTB domain 1 cysteine residue that are critical for Keap1 function, presumably as electrophile sensors (C151, C273, C288). Mutant forms of these residues diminish Keap1 repression of Nrf2 reporter activity in a dominant negative fashion but do not interfere with Keap1 dimerization.

### *Functionally validated NFE2L2 mutations*

The following table lists functionally validated amino acids/domains in Nrf2 based on review of the above literature. Mutations in these amino acids should lead to an increase in Nrf2 activity (**Table 1**).

**Table 1- Functionally important Nrf2 amino acids and mutations**

| NFE2L2 mutations | Domain | Function | Validation |
|---|---|---|---|
| aa.17-36 | DLG motif | Keap1 binding site | *in silico and in vitro* |
| aa.76-82 | ETGE motif | Keap1 binding site | *in silico and in vitro* |
| K44, K50, K52, K53, K56, K64, K68 | Lysine ubiquitination targets | Nrf2 degradation | *in silico and in vitro* |
| Exon 2 deletion | DLG/ETGE motifs | Keap1 binding site | *in vitro* |

Interestingly, a review of the TCGA squamous cell lung cancer (SQCLC) dataset and our own sequencing data show that virtually all mutations in *NFE2L2* occur in these validated regions as shown in **Table 2**.

**Table 2- *NFE2L2* mutations in SQCLCs (TCGA and MSK internal data)**

| ID | NFE2L2 mutation | Functional domain | Validated? |
|---|---|---|---|
| SQ-MAP | Q51H | alpha helix | No |
| TCGA-34-2608 | D27H | DLG motif | Yes |
| TCGA-34-2596 | D29G | DLG motif | Yes |
| TCGA-46-3768 | D29H | DLG motif | Yes |
| TCGA-34-5240 | D29H | DLG motif | Yes |
| TCGA-66-2783 | D29N | DLG motif | Yes |

| | | | |
|---|---|---|---|
| TCGA-33-4586 | D29Y | DLG motif | Yes |
| TCGA-22-5482 | F37del | DLG motif | Yes |
| TCGA-18-3415 | G31A | DLG motif | Yes |
| TCGA-66-2766 | G31A | DLG motif | Yes |
| TCGA-51-4081 | L30F | DLG motif | Yes |
| TCGA-46-3765 | L30F | DLG motif | Yes |
| TCGA-37-4135 | Q26L | DLG motif | Yes |
| TCGA-22-5473 | Q26P | DLG motif | Yes |
| TCGA-18-3411 | R34G | DLG motif | Yes |
| TCGA-34-5236 | R34P | DLG motif | Yes |
| TCGA-18-4721 | R34Q | DLG motif | Yes |
| TCGA-22-5477 | R34Q | DLG motif | Yes |
| TCGA-66-2795 | R34Q | DLG motif | Yes |
| TCGA-21-1071 | T80K | DLG motif | Yes |
| TCGA-34-2608 | W24C | DLG motif | Yes |
| SQ-MAP | R34P | DLG motif | Yes |
| SQ-MAP | G31A | DLG motif | Yes |
| SQ-MAP | G10R | DLG motif | No |
| SQ-MAP | G31R | DLG motif | Yes |
| SQ-MAP | W24S | DLG motif | Yes |
| SQ-MAP | P30_31 insIDL | DLG motif | Yes |
| SQ-MAP | L23del | DLG motif | Yes |
| SQ-MAP | R34G | DLG motif | Yes |
| SQ-MAP | D29H | DLG motif | Yes |
| SQ-MAP | D27G | DLG motif | Yes |
| SQ-MAP | R34L | DLG motif | Yes |
| TCGA-18-5592 | D77G | ETGE motif | Yes |
| TCGA-56-5897 | E79Q | ETGE motif | Yes |
| TCGA-66-2770 | E79Q | ETGE motif | Yes |
| TCGA-22-5474 | E79Q | ETGE motif | Yes |
| TCGA-39-5022 | E79Q | ETGE motif | Yes |
| TCGA-60-2709 | G81S | ETGE motif | Yes |
| TCGA-66-2787 | G81S | ETGE motif | Yes |
| TCGA-51-4079 | G81V | ETGE motif | Yes |
| SQ-MAP | E79Q | ETGE motif | Yes |
| SQ-MAP | E79K | ETGE motif | Yes |
| SQ-MAP | E79Q | ETGE motif | Yes |
| SQ-MAP | E79Q | ETGE motif | Yes |
| SQ-MAP | K487E | Neh1 | No |
| SQ-MAP | V105del | Neh2 | No |

### *Functionally validated KEAP1 mutations*

The following table lists the functionally validated amino acids/domains in Keap1 based on literature review. Mutations in these amino acids should lead to a loss of Keap1 function and increased Nrf2 activity (**Table 3**).

| **Table 3 Eligible and functionally validated Keap1 amino acids/mutations** | | | |
|---|---|---|---|
| **KEAP1 mutation sites** | **Domain** | **Function** | **Validation** |
| R380, R415, R483 | DGR-CTR | Nrf2 binding residues | *in silico and in vitro* |
| Y525, Q530, N382, Y573, F577, Y334, S363, S602, S555 | DGR-CTR | Nrf2 binding cavity | *in silico and in vitro* |
| G364C, G430C | DGR-CTR | Nrf2 binding cavity | *in silico and in vitro* |
| aa.315-624 | DGR-CTR | Nrf2 binding domain | *in silico* |
| aa.96-100 | BTB HKVVL | Keap1 dimerization | *in silico and in vitro* |
| C151, V155, V167 | BTB | electrophile sensor | *in vitro* |
| D244, S243, C273, C288 | IVR | electrophile sensor | *in vitro* |
| Any frameshift or nonsense mutation | N/A | N/A | N/A |

Review of the TCGA SQCLC data and our own internal sequencing data show that mutations in functionally validated amino acid positions comprise 21% of all *KEAP1* mutations, including nonsense and frameshifts (**Table 4**).

| **Table 4- *KEAP1* mutations in SQCLCs (TCGA and MSK internal data)** | | | |
|---|---|---|---|
| **ID** | **KEAP1 mutation** | **Functional domain** | **Validated?** |
| SQ-MAP | Q563* | DGR-CTR | Yes/truncation |
| SQ-MAP | E213* | IVR | Yes/truncation |
| SQ-MAP | R260* | IVR | Yes/truncation |
| TCGA-46-6025 | Q75* | BTB | Yes/truncation |
| SQ-MAP | I519fs | DGR-CTR | Yes/frame shift |
| TCGA-33-4532 | N469fs | DGR-CTR | Yes/frame shift |
| SQ-MAP | E205fs | IVR | Yes/frame shift |
| SQ-MAP | G430C | DGR-CTR | Yes |
| SQ-MAP | Y572C | DGR-CTR | Yes |
| SQ-MAP | L153F | BTB | No |
| SQ-MAP | S102L | BTB | No |
| SQ-MAP | S144Y | BTB | No |
| TCGA-18-3407 | V155F | BTB | No |
| TCGA-22-4599 | V155F | BTB | No |
| TCGA-43-2578 | V167F | BTB | No |
| TCGA-21-1077 | D422N | DGR-CTR | No |
| SQ-MAP | E488D | DGR-CTR | No |
| TCGA-18-5595 | E493D | DGR-CTR | No |

| | | | |
|---|---|---|---|
| TCGA-39-5036 | G423V | DGR-CTR | No |
| TCGA-37-5819 | G480W | DGR-CTR | No |
| TCGA-60-2722 | G480W | DGR-CTR | No |
| TCGA-43-6143 | I506V | DGR-CTR | No |
| SQ-MAP | M456V | DGR-CTR | No |
| SQ-MAP | N414I | DGR-CTR | No |
| TCGA-18-3409 | P318L | DGR-CTR | No |
| SQ-MAP | R320L | DGR-CTR | No |
| SQ-MAP | R320L | DGR-CTR | No |
| TCGA-66-2773 | R320Q | DGR-CTR | No |
| SQ-MAP | R326C | DGR-CTR | No |
| TCGA-66-2773 | R470C | DGR-CTR | No |
| TCGA-60-2710 | R470C | DGR-CTR | No |
| SQ-MAP | T609S | DGR-CTR | No |
| TCGA-66-2756 | V369L | DGR-CTR | No |
| TCGA-51-4081 | V418L | DGR-CTR | No |
| TCGA-66-2754 | W544C | DGR-CTR | No |
| SQ-MAP | H311R | IVR | No |
| TCGA-60-2723 | L231V | IVR | No |
| TCGA-33-4538 | L310P | IVR | No |
| SQ-MAP | R204L | IVR | No |
| TCGA-18-5595 | R260Q | IVR | No |
| TCGA-66-2777 | S224Y | IVR | No |
| TCGA-37-4133 | S243C | IVR | No |
| TCGA-39-5031 | R15L | NTR | No |

**Functional *KRAS* mutations**

*KRAS* mutations occur in approximately 25% of all non-squamous NSCLCs. Nearly all *KRAS* mutations occur in codons 12 and 13. Mutations in these codons lead to constitutive activation of KRAS by inhibiting its GTPase activity. This leads to activation of the MAP and PI3K/mTOR pathways.[9] Induction of mutant *KRAS* isoforms is oncogenic, and has formed the basis of many of the transgenic lung cancer models in use today.[10] These include models that have demonstrated, as noted above, dependence on the mTOR pathway and glutamine metabolism in the context of KEAP1 loss of function.[11] The below table lists the frequency breakdown of common *KRAS* mutations in NSCLC based on MSK-IMPACT sequencing data from 1,668 patients:

| Mutation | Frequency (N=458 KRAS mutations) |
|---|---|
| G12C | 42% |
| G12V | 15% |

| | |
|---|---|
| G12D | 13% |
| G12A | 9% |
| G13D | 4% |
| Q61H | 4% |
| G13C | 3.5% |
| G12S | 2.4% |

*KEAP1* co-alterations occur in 23% of all *KRAS* mutant lung cancer cases in the MSK-IMPACT dataset, circumscribing a sizable population of patients with both alterations. Specific *KRAS* mutations associated with *KEAP1* alterations include codon 12 alterations (G12A,C,S,V,D), codon 13 alterations (G13C,D,R) and codon 61 (Q61H).

***Integral Biomarker Inclusion Criteria***

Based on the preceding analysis, virtually all *NFE2L2* mutations detected to date are predicted to be functionally relevant. This clustering of mutations in specific subdomains of a larger regulatory domain is reminiscent of hotspot mutations in other oncogenes. Only mutations that occur in amino acids listed in **Table 1** should be included in the proposed phase 2 study of MLN0128 (TAK-228).

Eligible *KEAP1* mutations will be prioritized, but not limited, to those alterations that occur in the amino acids listed in **Table 3**.

Eligible *KRAS* mutations will include any missense mutation that occurs in codons 12, 13, and 61.

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## APPENDIX C BIOASSAY TEMPLATES

The below Appendix C describes the validation studies for the integral *NFE2L2* and *KEAP1* mutation biomarker testing. The validation study for *KRAS* mutations was previously published as part of the MSK-IMPACT validation study leading to its FDA approval in 2017 as an in vitro diagnostic test to identify genetic mutations across cancers. Please refer to Cheng DT et al. J Mol Diagn 2015 17(3):251-64 (PMID25801521) for the validation study details.

### Validation Study of *KEAP1* and *NFE2L2* Mutations

**Accuracy Study**

To carry out this validation, we obtained a total of 23 positive tumor DNA samples (18 *KEAP1* and 5 *NFE2L2*) that had been previously genotyped by our "MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets): A Hybridization-Capture Based Next Generation Sequencing Assay". The samples were also studied by Sanger DNA sequencing. All DNA sequencing reactions were performed in both forward and reverse directions.

Concordant results were obtained for all 23 samples by DNA sequencing and MSK-IMPACT assay. The results of the validation study are summarized in **Tables 1 & 2**.

#### 1) Accuracy study – *KEAP1* mutations

**Table 1.A**: Sample coverage (mean coverage across all exons) and total number of mutations called across all 340 genes, for samples used in validation of this clinical exon.

| | Sample ID# | Sample Coverage | Total Number of Mutations called across all 340 genes | Normal sample used |
|---|---|---|---|---|
| **1** | 2242-14 | 866 | 15 | Matched |
| **2** | 4488-14 | 526 | 5 | Matched |
| **3** | 4673-14 | 1149 | 8 | Matched |
| **4** | 4756-14 | 749 | 11 | Matched |
| **5** | 4869-14 | 467 | 8 | Matched |
| **6** | 4930-14 | 172 | 7 | Matched |
| **7** | 5566-14 | 654 | 9 | Matched |
| **8** | 6134-14 | 897 | 21 | Matched |
| **9** | 6451-14 | 729 | 39 | Matched |
| **10** | 6714-14 | 591 | 9 | Matched |
| **11** | 6989-14 | 540 | 11 | Matched |
| **12** | 7327-14 | 422 | 19 | Matched |
| **13** | 7447-14 | 143 | 15 | Matched |
| **14** | 7909-14 | 667 | 8 | Matched |
| **15** | 8091-14 | 365 | 8 | Matched |
| **16** | 8707-14 | 508 | 4 | Matched |
| **17** | 8731-14 | 714 | 6 | Matched |
| **18** | 8831-14 | 1020 | 9 | Matched |

**Table 1.B**: List of mutations detected that correspond to the known *KEAP1* variants for the tested samples.

| | Sample ID# | Gene | Exon | cDNA change | AA change | DP | AD | VF | Normalized Coverage |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 2242-14 | KEAP1 | exon4 | c.1511A>G | p.N504S | 813 | 125 | 0.15 | 0.9 |
| **2** | 4488-14 | KEAP1 | exon6 | c.1801C>T | p.R601W | 517 | 167 | 0.32 | 1 |
| **3** | 4673-14 | KEAP1 | exon2 | c.295G>A | p.V99M | 946 | 395 | 0.42 | 0.8 |
| **4** | 4756-14 | KEAP1 | exon5 | c.1626_1627delGA | p.E542fs | 801 | 247 | 0.31 | 1.1 |
| **5** | 4869-14 | KEAP1 | exon4 | c.1486G>T | p.E496X | 886 | 223 | 0.25 | 1.9 |
| **6** | 4930-14 | KEAP1 | exon3 | c.932A>G | p.H311R | 92 | 39 | 0.42 | 0.5 |
| **7*** | 5566-14a | KEAP1 | exon2 | c.16A>T | p.R6W | 153 | 55 | 0.36 | 0.2 |
| | 5566-14b | KEAP1 | exon3 | c.994G>T | p.G332C | 402 | 127 | 0.32 | 0.6 |
| **8** | 6134-14 | KEAP1 | exon2 | c.592G>T | p.E198X | 1020 | 250 | 0.25 | 1.1 |
| **9** | 6451-14 | KEAP1 | exon3 | c.997G>T | p.G333C | 661 | 462 | 0.7 | 0.9 |
| **10** | 6714-14 | KEAP1 | exon3 | c.655G>A | p.E219K | 486 | 160 | 0.33 | 0.8 |
| **11*** | 6989-14a | KEAP1 | exon2 | c.411T>G | p.I137M | 581 | 69 | 0.12 | 1.1 |
| | 6989-14b | KEAP1 | exon2 | c.414_424delATTCGCCTACA | p.E138fs | 634 | 69 | 0.11 | 1.2 |
| | 6989-14c | KEAP1 | exon3 | c.1103G>T | p.C368F | 647 | 152 | 0.23 | 1.2 |
| **12** | 7327-14 | KEAP1 | exon5 | c.1638delC | p.F546fs | 507 | 218 | 0.43 | 1.2 |
| **13** | 7447-14 | KEAP1 | exon2 | c.244C>T | p.Q82X | 99 | 23 | 0.23 | 0.7 |
| **14** | 7909-14 | KEAP1 | exon6 | c.1808G>T | p.G603V | 637 | 274 | 0.43 | 1 |
| **15** | 8091-14 | KEAP1 | exon3 | c.1006C>T | p.R336X | 245 | 51 | 0.21 | 0.7 |
| **16** | 8707-14 | KEAP1 | exon3 | c.1085G>C | p.R362P | 418 | 140 | 0.33 | 0.8 |
| **17** | 8731-14 | KEAP1 | exon3 | c.772G>T | p.E258X | 491 | 100 | 0.2 | 0.7 |
| **18*** | 8831-14a | KEAP1 | exon2 | c.493G>A | p.D165N | 1041 | 362 | 0.35 | 1 |
| | 8831-14b | KEAP1 | exon2 | c.574G>A | p.E192K | 1006 | 348 | 0.35 | 1 |

*These samples had more than 1 mutation

## 2) Accuracy study – *NFE2L2* mutations

**Table 2.A**: Sample coverage (mean coverage across all exons) and total number of mutations called across all 340 genes, for samples used in validation of this clinical exon.

| | Sample ID# | Sample Coverage | Total Mutations | Normal sample used |
|---|---|---|---|---|
| **1** | 5155-14 | 826 | 10 | Matched |
| **2** | 7894-14 | 650 | 10 | Matched |
| **3** | 8045-14 | 1254 | 3 | Matched |
| **4** | 8556-14 | 1112 | 11 | Matched |
| **5** | 8586-14 | 920 | 56 | Matched |

**Table 2.B**: List of mutations detected that correspond to the known *NFE2L2* variants for the tested samples.

| | Sample ID# | Gene | Exon | cDNA change | AA change | DP | AD | VF | Normalized Coverage |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 5155-14 | NFE2L2 | exon2 | c.75_76delinsAA | p.Q26K | 1261 | 716 | 0.57 | 1.5 |
| **2** | 7894-14 | NFE2L2 | exon2 | c.100C>G | p.R34G | 1279 | 120 | 0.09 | 2 |
| **3** | 8045-14 | NFE2L2 | exon2 | c.76C>A | p.Q26K | 1213 | 471 | 0.39 | 1 |
| **4** | 8556-14 | NFE2L2 | exon2 | c.100C>G | p.R34G | 1639 | 303 | 0.18 | 1.5 |
| **5** | 8586-14 | NFE2L2 | exon2 | c.88C>T | p.L30F | 1378 | 254 | 0.18 | 1.5 |

**Precision and Reproducibility Study**

The precision and reproducibility studies were extensively performed in the initial MSK-IMPACT validation according to NGS NYSDOH guidelines (attached). In addition, two DNA samples, one *KEAP1* positive and one *NFE2L2* positive, were studied in triplicate in the same run for the intra-assay precision study. The same DNA samples were also studied over three different runs for the inter-assay reproducibility study. Concordant results were obtained for both intra- and inter-assay reproducibility studies**.** Replicates tested within the same run were assigned different barcodes to ensure reproducibility of results regardless of sample/barcode combination. Each replicate was assigned a different barcode (bc). Differences in barcodes, coverage depth, allele depth (AD) and variant frequency (VF) for the known variant across intra- and inter- run replicates are listed in **Tables 3.1** & **3.2**.

### 3.1) Precision and reproducibility studies of *KEAP1*

Table 3.1A: Total number of mutations called across all 340 genes, for *KEAP1* intra- (precision) and inter-run (reproducibility) replicates.

| Sample | Total Number of Mutations called across all 340 genes | Normal sample used |
|---|---|---|
| 7555-13-1A | 13 | Matched |
| 7555-13-1B | 13 | Matched |
| 7555-13-1C | 13 | Matched |
| 7555-13-2 | 13 | Matched |
| 7555-13-3 | 13 | Matched |

Table 3.1B-i: Differences in Illumina TruSeq barcode, coverage, allele depth and variant frequency for the known variant detected in each inter-run (reproducibility) replicate.

| Sample | cDNA change | AAchange | Validation Run | Barcode | Coverage | AD | VF | Normalized DP |
|---|---|---|---|---|---|---|---|---|
| 7555-13-1A | c.811G>A | p.V271M | KEAP1 Intra | bc34 | 910 | 483 | 0.42 | 1.26 |
| 7555-13-1B | c.811G>A | p.V271M | KEAP1 Intra | bc36 | 1011 | 506 | 0.42 | 1.19 |
| 7555-13-1C | c.811G>A | p.V271M | KEAP1 Intra | bc38 | 1185 | 688 | 0.44 | 1.33 |

Table 3.1B-ii: Differences in Illumina TruSeq barcode, coverage, allele depth and variant frequency for the known variant detected in each intra-run (precision) replicate.

| Sample | cDNA change | AAchange | Validation Run | Barcode | Coverage | AD | VF | Normalized DP |
|---|---|---|---|---|---|---|---|---|
| 7555-13-1A | c.811G>A | p.V271M | KEAP1 Inter | bc34 | 910 | 483 | 0.42 | 1.26 |
| 7555-13-2 | c.811G>A | p.V271M | KEAP1 Inter | bc24 | 1078 | 517 | 0.40 | 1.19 |
| 7555-13-3 | c.811G>A | p.V271M | KEAP1 Inter | bc43 | 1044 | 560 | 0.43 | 1.25 |

## 3.2) Precision and reproducibility studies of *NFE2L2*

Table 3.2A: Total number of mutations called across all 340 genes, for *NFE2L2* intra- (precision) and inter-run (reproducibility) replicates.

| Sample | Total Number of Mutations called across all 340 genes | Normal sample used |
|---|---|---|
| 5155-14-1A | 10 | Matched |
| 5155-14-1B | 10 | Matched |
| 5155-14-1C | 10 | Matched |
| 5155-14-2 | 10 | Matched |
| 5155-14-3 | 10 | Matched |

Table 3.2B-i: Differences in Illumina TruSeq barcode, coverage, allele depth and variant frequency for the known variant detected in each inter-run (reproducibility) replicate.

| | cDNA change | AA change | Validation Run | Barcode | Coverage | AD | VF | Normalized DP |
|---|---|---|---|---|---|---|---|---|
| 5155-14-1A | c.76C>A | p.Q26K | NEF2L2 Intra | bc39 | 702 | 555 | 0.53 | 1.51 |
| 5155-14-1B | c.76C>A | p.Q26K | NEF2L2 Intra | bc40 | 693 | 537 | 0.51 | 1.51 |
| 5155-14-1C | c.76C>A | p.Q26K | NEF2L2 Intra | bc42 | 621 | 491 | 0.52 | 1.53 |

Table 2.2B-ii: Differences in Illumina TruSeq barcode, coverage, allele depth and variant frequency for the known variant detected in each intra-run (precision) replicate.

| | cDNA change | AA change | Validation Run | Barcode | Coverage | AD | VF | Normalized DP |
|---|---|---|---|---|---|---|---|---|
| 5155-14-1A | c.76C>A | p.Q26K | NEF2L2 Inter | bc39 | 702 | 555 | 0.53 | 1.51 |
| 5155-14-2 | c.76C>A | p.Q26K | NEF2L2 Inter | bc38 | 742 | 545 | 0.51 | 1.45 |
| 5155-14-3 | c.76C>A | p.Q26K | NEF2L2 Inter | bc43 | 596 | 462 | 0.55 | 1.42 |

## APPENDIX D PATIENT PILL DIARY

A Study to Test if MLN0128 (TAK-228) can Treat Patients with Non-Small Cell Lung Cancers with *NFE2L2* and *KEAP1* Mutations

**Name:_____** **MRN:_____**

**To be completed by NP:**

Number of pills dispensed:_____ 1mg___ 3mg_____ Date: _____

Daily dose of MLN0128 (TAK-228):_____mg Cycle :_____

Pill bottle returned: circle Yes or N

**Instructions for patient:**

1. You will be given a pill diary to record the time and doses of MLN0128 (TAK-228) you take each day. This diary along with any leftover pills and the pill bottles should be brought to each visit with your study doctor.
2. MLN0128 (TAK-228) should be taken once daily, at approximately the same time every day.
3. Take MLN0128 (TAK-228) on an empty stomach at least 2 hours after a meal. Do not eat or drink (except water) for at least 1 hour after taking MLN0128 (TAK-228).
4. Each dose should be taken with a glass of water and consumed whole (no chewing). The capsules should not be opened, chewed, or crushed. If multiple capsules are required for each dose, they should be taken at the same time.
5. If you miss a dose, you can still take that dose if it has been less than 12 hours since you missed the dose.
6. If you vomit after taking the pill, do not replace that dose. If you can see that the pill has come up, mark the dose as missed in the comments.

| Cycle___ | Date | Time (circle one) | Amount (mg) | Comments |
|---|---|---|---|---|
| Day 1 | | AM/PM | | |
| Day 2 | | AM/PM | | |
| Day 3 | | AM/PM | | |
| Day 4 | | AM/PM | | |
| Day 5 | | AM/PM | | |
| Day 6 | | AM/PM | | |
| Day 7 | | AM/PM | | |
| Day 8 | | AM/PM | | |
| Day 9 | | AM/PM | | |
| Day 10 | | AM/PM | | |
| Day 11 | | AM/PM | | |
| Day 12 | | AM/PM | | |
| Day 13 | | AM/PM | | |
| Day 14 | | AM/PM | | |
| Day 15 | | AM/PM | | |
| Day 16 | | AM/PM | | |
| Day 17 | | AM/PM | | |
| Day 18 | | AM/PM | | |
| Day 19 | | AM/PM | | |

| | | | | |
|---|---|---|---|---|
| **Day 20** | | AM/PM | | |
| **Day 21** | | AM/PM | | |
| **Day 22** | | AM/PM | | |
| **Day 23** | | AM/PM | | |
| **Day 24** | | AM/PM | | |
| **Day 25** | | AM/PM | | |
| **Day 26** | | AM/PM | | |
| **Day 27** | | AM/PM | | |
| **Day 28** | | AM/PM | | |

Patient signature:_____ Date:__/__/__

Consenting professional / NP signature:_____Date:__/__/__

## APPENDIX E PATIENT GLUCOSE MONITORING LOG

A Study to Test if MLN0128 (TAK-228) can Treat Patients with Non-Small Cell Lung Cancers with *NFE2L2* and *KEAP1* Mutations

Patient Name:_____

Patient MRN:_____

**Directions:**

- You will need to perform the glucose monitoring test on a daily basis, predose on dosing days, and at approximately the same time each day- prior to breakfast. **You are required to fast for at least 8 hours prior to testing.** Fasting means not eating or drinking any liquids (with the exception of water).

- In the log, please write in the date, note the time you completed the glucose test, whether you fasted or not (fed state) before the test, and your glucose levels as provided by your glucometer.

- Please 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.

Site Contact Name:_____

Site Contact: Number:_____

| Day | Date | Approximate time of Test (Circle AM or PM) | Fasting or Fed (Circle One) | Glucose Test Results (mg/dL) |
|---|---|---|---|---|
| **Day 1** | | ____:____ am / pm | Fasting or Fed | |
| **Day 2** | | ____:____ am / pm | Fasting or Fed | |
| **Day 3** | | ____:____ am / pm | Fasting or Fed | |
| **Day 4** | | ____:____ am / pm | Fasting or Fed | |
| **Day 5** | | ____:____ am / pm | Fasting or Fed | |

| | | | | |
|---|---|---|---|---|
| **Day 6** | | ____:____ am / pm | Fasting or Fed | |
| **Day 7** | | ____:____ am / pm | Fasting or Fed | |
| **Day 8** | | ____:____ am / pm | Fasting or Fed | |
| **Day 9** | | ____:____ am / pm | Fasting or Fed | |
| **Day 10** | | ____:____ am / pm | Fasting or Fed | |
| **Day 11** | | ____:____ am / pm | Fasting or Fed | |
| **Day 12** | | ____:____ am / pm | Fasting or Fed | |
| **Day 13** | | ____:____ am / pm | Fasting or Fed | |
| **Day 14** | | ____:____ am / pm | Fasting or Fed | |
| **Day 15** | | ____:____ am / pm | Fasting or Fed | |
| **Day 16** | | ____:____ am / pm | Fasting or Fed | |
| **Day 17** | | ____:____ am / pm | Fasting or Fed | |
| **Day 18** | | ____:____ am / pm | Fasting or Fed | |
| **Day 19** | | ____:____ am / pm | Fasting or Fed | |
| **Day 20** | | ____:____ am / pm | Fasting or Fed | |
| **Day 21** | | ____:____ am / pm | Fasting or Fed | |
| **Day 22** | | ____:____ am / pm | Fasting or Fed | |
| **Day 23** | | ____:____ am / pm | Fasting or Fed | |
| **Day 24** | | ____:____ am / pm | Fasting or Fed | |
| **Day 25** | | ____:____ am / pm | Fasting or Fed | |
| **Day 26** | | ____:____ am / pm | Fasting or Fed | |
| **Day 27** | | ____:____ am / pm | Fasting or Fed | |
| **Day 28** | | ____:____ am / pm | Fasting or Fed | |

Patient Signature:_____ Date:_____

Investigator Signature:_____ Date:_____

## APPENDIX F LIST OF RELEVANT CYTOCHROME P450 INHIBITORS AND INDUCERS

| | | |
|---|---|---|
| **Moderate CYP1A2 Inhibitors** | | |
| Cimetidine | methoxsalen | |
| **Strong CYP1A2 Inhibitors** | | |
| Fluvoxamine | Ciprofloxacin | |
| **Clinically Significant Enzyme Inducers** | | |
| Carbamazepine | rifabutin | St. John's Wort |
| Phenobarbital | rifampin | Phenytoin |
| rifapentine | | |

Source:
fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ ucm093664.htm.
Note that these lists are not exhaustive.