RESEARCH ARTICLE

TAK-228 (formerly MLN0128), an investigational oral dual TORC1/2 inhibitor: A phase I dose escalation study in patients with relapsed or refractory multiple myeloma, non-Hodgkin lymphoma, or Waldenström's macroglobulinemia

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The PI3K/AKT/mTOR signaling pathways are frequently dysregulated in multiple human cancers, including multiple myeloma (MM), non-Hodgkin lymphoma (NHL), and Waldenström's macroglobulinemia (WM). This was the first clinical study to evaluate the safety, tolerability, maximal-tolerated dose (MTD), dose-limiting toxicity (DLT), pharmacokinetics, and preliminary clinical activity of TAK-228, an oral TORC1/2 inhibitor, in patients with MM, NHL, or WM. Thirty-nine patients received TAK-228 once daily (QD) at 2, 4, 6, or 7 mg, or QD for 3 days on and 4 days off each week (QDx3d QW) at 9 or 12 mg, in 28-day cycles. The overall median age was 61.0 years (range 46–85); 31 patients had MM, four NHL, and four WM. Cycle 1 DLTs occurred in five QD patients (stomatitis, urticaria, blood creatinine elevation, fatigue, and nausea and vomiting) and four QDx3d QW patients (erythematous rash, fatigue, asthenia, mucosal inflammation, and thrombocytopenia). The MTDs were determined to be 4 mg QD and 9 mg QDx3d QW. Thirty-six patients (92%) reported at least one drug-related toxicity; the most common grade ≥3 drug-related toxicities were thrombocytopenia (15%), fatigue (10%), and neutropenia (5%). TAK-228 exhibited a dose-dependent increase in plasma exposure and no appreciable accumulation with repeat dosing; mean plasma elimination half-life was 6–8 hr. Of the 33 response-evaluable patients, one MM patient had a minimal response, one WM patient achieved partial response, one WM patient had a minor response, and 18 patients (14 MM, two NHL, and two WM) had stable disease. These findings encourage further studies including combination strategies.
Am. J. Hematol. 91:400–405, 2016.

Introduction

The mammalian target of rapamycin (mTOR) regulates tumor cell growth, metabolism, and motility [1,2]. mTOR operates in two distinct multiprotein complexes, target of rapamycin complex 1 and 2 (TORC1 and TORC2), in the canonical PI3K/AKT/mTOR pathway [3]. Both complexes are stimulated by growth factors; TORC1 regulates cell growth by controlling S6 kinase (S6K) and eIF-4E-binding protein 1 (4E-BP1) activity while TORC2 promotes cellular survival through activation of kinases, such as AKT kinase [3–5].

The PI3K/AKT/mTOR signaling pathways are frequently dysregulated in multiple human cancers, including multiple myeloma (MM), non-Hodgkin lymphoma (NHL), and Waldenström's macroglobulinemia (WM) [1,2,6–11]. Constitutive activation of the mTOR pathway has been demonstrated in MM cell lines and primary patient samples [12]. Similar to MM cell lines, primary WM cells have been shown to have constitutively activated PI3K/AKT signaling that contribute to enhanced mTOR activation [13].

Rapamycin and its analogs (rapalogs) have demonstrated promising efficacy against renal cell carcinoma and advanced pancreatic neuroendocrine tumors [14,15] and are under investigation for other malignancies [16,17]. However, these agents function as allosteric inhibitors of TORC1 and even at high concentrations only partially inhibit TORC2 in most tumor cells, often leaving the signaling cascades downstream of both TORC1/2 complexes active [18–20]. Under normal conditions, TORC1 activates S6K, which then inhibits insulin receptor substrate-1 (IRS-1); this feedback circuit subsequently prevents modulation of TORC2 and overstimulation of PI3K pathway [21]. Abrogation of this feedback circuit by rapamycin or rapalogs eventually results in increased phosphorylation of Akt and paradoxic hyperactive signaling, which can lead to enhanced survival and chemoresistance [22–26].

Additional Supporting Information may be found in the online version of this article.

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Contract grant sponsor: Millennium Pharmaceuticals, Inc.
Received for publication: 4 January 2016; **Revised:** 8 January 2016; **Accepted:** 12 January 2016
Am. J. Hematol. 91:400–405, 2016.
Published online: 22 January 2016 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.24300

Furthermore, dual TORC1/2 inhibition has been shown to be more active than TORC1 inhibition alone. In MM cells, inhibition of TORC1 and TORC2 functions using a dual inhibitor resulted in increased apoptosis compared to rapamycin [27]. Preclinical data in WM cells have shown constitutively activated PI3K/AKT signaling, a potential culprit in cellular resistance to rapalogs [13]. In patients with MM, everolimus and another rapalog, temsirolimus, have shown activity in combination with proteasome inhibitors but not as single agents [28,29]. Another study investigating single-agent everolimus has reported an overall response rate of 70% in patients with WM [30,31]. TAK-228 (formerly MLN0128/INK128) is an investigational, oral and selective adenosine triphosphate (ATP) site kinase inhibitor of both TORC1 and TORC2 [32,33]. TAK-228 has been shown to inhibit proliferation and induce apoptosis in preclinical models of B-cell acute lymphoblastic leukemia [33]. In MM cell lines and plasma cells from patients, TAK-228 inhibited both TORC1 and TORC2 activities, induced cell cycle arrest, and suppressed survival more than rapamycin [12].

Here, we present results from a phase I study (INK128-002, NCT01118689) evaluating TAK-228 (administered in two dosing schedules) in patients with relapsed or refractory MM, NHL, or WM. This study represents the first clinical report of TORC1/2 inhibition in MM and B-cell malignancies.

Methods

Patients. Eligible patients were aged ≥18 years and had relapsed/refractory MM with measurable serum/urine monoclonal protein or involved light chains ≥10 mg dL^{-1} (with abnormal serum-free light chain ratio or measurable plasmacytoma); NHL, without central nervous system involvement, and had previously failed or were not eligible for standard of care therapy; or relapsed/refractory WM with measurable IgM in the serum and detectable lymphoplasmacytic cells in the bone marrow (see Supporting Information for full eligibility criteria).

The study was approved by the institutional review board at each site and conducted per the Declaration of Helsinki, the International Conference on Harmonisation, and Good Clinical Practice guidelines. All patients provided written informed consent.

Study design. Two dosing schedules were investigated: oral TAK-228 once daily (QD at 2, 4, 6, or 7 mg) and QD for 3 days on/4 days off weekly (QDx3d QW at 9 or 12 mg), in 28-day cycles, for up to 1 year. Enrollment began with the QD dosing schedule and enrollment in the QDx3d QW cohorts proceeded in parallel, with random assignments to open cohorts. TAK-228 was administered in the morning with food. Prohibited concomitant medications are detailed in the Supporting Information.

The primary objective was to evaluate the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of TAK-228 in patients with MM, NHL, or WM (see Supporting Information for DLT definitions). Secondary objectives were to determine the pharmacokinetics (PK) of TAK-228, the pharmacodynamic (PD) effects of TAK-228 activity in peripheral blood cells, and antitumor activity of TAK-228. Dose was escalated within a dosing schedule using a modified Fibonacci schema with a standard 3 + 3 design, based on DLTs reported in cycle 1. The MTD was the highest TAK-228 dose level with six patients treated and ≤1 DLT during cycle 1. Intrapatient dose escalation was permitted, as described in Supporting Information Methods along with additional dose-modification guidelines.

Assessments. Safety, including daily fasting glucose levels, was assessed throughout treatment and until 30 days after the last dose of TAK-228 (Supporting Information Methods). Toxicities were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 4.0. Response was assessed using the International Myeloma Working Group uniform response criteria for MM [34] plus European Group for Blood and Marrow Transplantation criteria for minimal response [35], the International Working Group revised response criteria for NHL [36], and the updated Third International Workshop criteria for WM and for minor response [37,38]. Blood samples were collected at multiple timepoints pre- and post- TAK-228 dosing on day 1 in cycles 1 and 2 for PK assessment. The PD effects of TAK-228 activity were not formally analyzed due to insufficient sample quality and technical issues with the assays used (Supporting Information).

Statistical methods. All patients in the safety analyses received ≥1 dose of TAK-228. DLT-evaluable, response-evaluable, and PK-evaluable populations are defined in the Supporting Information. To determine MTD, patients were evaluated per the actual starting dose of TAK-228 during cycle 1. The PK parameters for TAK-228 were estimated using noncompartmental methods with WinNonlin® Professional V6.2 (Pharsight, Mountain View, CA) and summarized by descriptive statistics.

TABLE I. Patient Demographics and Baseline Characteristics

	Total; *N* = 39
Age, years, median (range)	61.0 (46–85)
Sex, *n* (%)	
Male	21 (54)
Female	18 (46)
Race, *n* (%)	
White	34 (87)
Asian	2 (5)
Black or African American	2 (5)
Unknown	1 (3)
Primary diagnosis, *n* (%)	
MM	31 (80)
Years since diagnosis, median (range)	4.6 (2–20)
ISS Staging, *n* (%)	
I	6 (19)
II	10 (32)
III	1 (3)
Unknown	14 (45)
NHL	4 (10)
Grade	
High	1 (25)
Low	3 (75)
Type	
Diffuse Large B Cell Lymphoma	2 (50)
Mantle Cell Lymphoma	1 (25)
Other	1 (25)
WM	4 (10)
Years since diagnosis, median (range)	7.2 (4–10)
IPSSWM Risk Category, *n* (%)	
Low	2 (50)
Intermediate	0
High	0
Unknown	1 (25)
Number of prior therapies, median (range)	3.0 (1–10)
Prior systemic treatments received, *n*[a] (%)	
Chemotherapy	31 (79)
Other	31 (79)
Immunomodulatory drugs	28 (72)
Steroid	27 (69)
Autologous transplant	19 (49)
Monoclonal antibody	9 (23)
Other small-molecule targeted therapy	8 (21)
Anti-angiogenic	3 (8)
Allogenic transplant	1 (3)

[a] Patients may be counted more than once.
IPSSWM, international prognostic scoring system for Waldenström macroglobulinemia; ISS, international staging system; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; WM, Waldenström's macroglobulinemia.

Results

A total of 39 patients were enrolled from six study sites in the United States between November 2010 and November 2012. The median age of all patients was 61.0 years (range 46–85); 80% ($n = 31$) of patients were diagnosed with MM, 10% ($n = 4$) with NHL, and 10% ($n = 4$) with WM. The patients' demographics and baseline characteristics are summarized in Table I. Patients discontinued TAK-228 due to one or more of the following reasons: disease progression ($n = 20$), adverse events (AEs) ($n = 10$), patient decision ($n = 11$), investigator decision ($n = 3$), and other ($n = 3$).

DLTs and MTD determination

Twenty-six patients were randomly assigned to the QD dosing schedule and 13 to QDx3d QW. Five patients in the QD dosing group were administered TAK-228 at 2 mg, seven at 4 mg, eight at 7 mg, and then, following dose de-escalation, six at 6 mg. In the QDx3d QW group, six patients received TAK-228 at 9 mg and seven received 12 mg.

Nineteen patients (73%) on the QD dosing schedule were DLT-evaluable; seven patients were not eligible for DLT evaluation because

they received <75% of the planned TAK-228 dosing in cycle 1 due to reasons that were not related to study drug (including AEs, noncompliance, or decision to discontinue early). All patients on QDx3d QW were DLT-evaluable. None of the three evaluable patients in the 2-mg QD group reported DLTs. In the successive 4-mg QD group, one of six evaluable patients experienced a DLT of grade 3 stomatitis from day 18 to 27 and was treated with magic mouthwash and moxifloxacin hydrochloride; enrollment proceeded to the next dose level. Next, at 7 mg QD, one of four evaluable patients experienced a DLT of grade 3 urticaria, and two patients experienced dose reductions during cycle 1 as a result of grade 2 fatigue and grade 1 dizziness, vomiting, and nausea, respectively. All AEs in these patients resolved without sequelae.

Based on these findings at 7 mg, the next dose level tested was 6 mg QD; three of six evaluable patients reported DLTs (one patient with grade 2 increased blood creatinine, one with grade 3 fatigue, and one with grade 2 nausea and vomiting). The increased blood creatinine level was reported on day 14 and TAK-228 dosing was interrupted until day 21. On day 28, the patient's blood creatinine level was still higher than baseline level; the patient discontinued TAK-228 dosing and received one subcutaneous dose of erythropoietin due to the AE. On day 31, the patient withdrew from the study. The event of grade 3 fatigue lasted from days 2 to 37. The patient discontinued TAK-228 as a result of this event, which resolved without sequelae. The single episode of grade 2 nausea and vomiting was reported on day 2 and TAK-228 dosing was interrupted. The AEs resolved without sequelae and the patient continued participation in the study. As a result of these DLTs, the MTD for the QD schedule was determined to be 4 mg.

On the QDx3d QW schedule, only one of six evaluable patients in the 9-mg group reported DLTs, of grade 3 erythematous rash on day 20 and grade 3 fatigue on days 20–27. The patient was discontinued from the study; the events were not treated with medication and resolved without sequelae on day 30.

At the next dose level of 12 mg, three of seven evaluable patients reported DLTs (one patient each with grade 3 asthenia, grade 3 mucosal inflammation, and grade 3 thrombocytopenia). The grade 3 asthenia lasted from day 22 to day 32, TAK-228 dosing was interrupted, and the AE resolved without sequelae. The event of grade 3 mucosal inflammation was reported on day 4, treated with magic mouthwash, and resolved without sequelae on day 9. The patient withdrew from the study; the last dose of TAK-228 was administered on day 3. TAK-228 dosing was also interrupted in the patient who experienced grade 3 thrombocytopenia on days 15–28 that resolved without sequelae. The MTD for QDx3d QW was therefore determined to be 9 mg.

Treatment

All patients received at least one dose of TAK-228 Patients on the QD dosing schedule received a median of 2 treatment cycles (range 1–12) of TAK-228, with one patient receiving 8 cycles and one patient receiving 12 cycles. Median treatment duration was 7.6 weeks (range 0.4–49.6), and median cumulative dose of TAK-228 was 153 mg (range 6–1,480).

Patients on the QDx3d QW dosing schedule received a median of three cycles (range 1–15), with one patient receiving 9 cycles and one patient receiving 15 cycles. Median treatment duration was 10.1 weeks (range 0.4–59.0), and median cumulative dose was 324 mg (range 36–1,602). One patient had a dose escalation from 4 mg QD to 7 mg QD starting at cycle 7 week 1 and continuing through the last dose (cycle 8, week 2). This patient is included in the 4-mg dose group for all safety analyses. No other patient had a dose escalation.

Adverse events

All patients ($n = 39$) reported at least one any-cause AE and 92% of patients reported at least one AE considered related to TAK-228.

TABLE II. Safety Profiles and Summary of Most Common TAK-228-related AEs (Any Grade Occurring in ≥10% of Patients Overall), by TAK-228 Dosing Schedule

Patients, *n* (%)	QD; *N* = 26	QDx3d QW; *N* = 13	Total; *N* = 39
Any AE	26 (100)	13 (100)	39 (100)
Any drug-related AE	23 (88)	13 (100)	36 (92)
Any serious AE	7 (27)	4 (31)	11 (28)
Any drug-related serious AE	0	1 (8)	1 (3)
Any AE leading to TAK-228 discontinuation	11 (42)	3 (23)	14 (36)
Any AE leading to TAK-228 dose modification or interruption	13 (50)	9 (69)	22 (56)
On study deaths	2 (8)	0	2 (5)
Most common drug-related AEs			
Nausea	12 (46)	7 (54)	19 (49)
Fatigue	8 (31)	8 (62)	16 (41)
Hyperglycemia	9 (35)	6 (46)	15 (38)
Thrombocytopenia	7 (27)	5 (38)	12 (31)
Rash[a]	4 (15)	3 (23)	7 (18)
Anemia	4 (15)	3 (23)	7 (18)
Decreased appetite	4 (15)	3 (23)	7 (18)
Stomatitis	5 (19)	2 (15)	7 (18)
Vomiting	4 (15)	3 (23)	7 (18)
Diarrhea	3 (12)	3 (23)	6 (15)
Constipation	4 (15)	1 (8)	5 (13)
Hypophosphatemia	3 (12)	2 (15)	5 (13)
White blood cell count decreased	4 (15)	1 (8)	5 (13)
Blood creatinine increased	3 (12)	1 (8)	4 (10)
Hypomagnesemia	3 (12)	1 (8)	4 (10)
Neutropenia	0	4 (31)	4 (10)

[a] Rash = rash, rash erythematous, rash macular, and rash pruritic.
AE, adverse event; QD, once daily; QDx3d QW, QD for 3 days on and 4 days off each week.

Safety profiles and most common AEs considered related to TAK-228 are summarized by dosing schedule and for all patients in Table II.

On the QD dosing schedule ($n = 26$), 88% of patients reported at least one AE that was considered related to TAK-228. The most common drug-related AEs include: nausea (46%), hyperglycemia (35%), fatigue (31%), and thrombocytopenia (27%). Common AEs appeared more frequent at higher doses of TAK-228. Six and five patients (100 and 63%) at the 6- and 7-mg doses, respectively, reported nausea compared to 1 patient (20%) at the 2-mg dose. Three and five patients (50% and 63%) at the 6- and 7-mg doses, respectively, experienced hyperglycemia compared to one patient (20%) at the 2-mg dose. Thirty-one percent of patients on the QD dosing schedule reported at least one grade ≥3 AE considered related to TAK-228, with the most frequent AE being thrombocytopenia (8%) (Table III).

The most common any-cause AEs on the QD dosing schedule included: nausea (50%), fatigue (42%), hyperglycemia (35%), and thrombocytopenia (31%) (Supporting Information Table I); 54% of patients had any-cause grade ≥3 AEs and the most common AE was thrombocytopenia (12%) (Supporting Information Table II). Overall, 27% of patients on the QD dosing schedule had a serious AE (SAE), none of which were considered related to TAK-228. Two patients died within 30 days of their last dose of TAK-228; one due to subdural hemorrhage and one due to progressive disease, these events were not considered related to treatment with TAK-228.

On the QD × 3d QW dosing schedule, all patients ($n = 13$) reported at least one AE considered related to TAK-228 (Table II). The most common drug-related AEs include: fatigue (62%), nausea (54%), hyperglycemia (46%), and thrombocytopenia (38%). Drug-related grade ≥3 AEs were reported in 77% of patients; the most common AEs were: thrombocytopenia (31%), fatigue (23%), and neutropenia (15%) (Table III). The most common any-cause AEs include: nausea (69%), fatigue (62%), and hyperglycemia and thrombocytopenia

 doi:10.1002/ajh.24300

TABLE III. Summary of Grade ≥3 TAK-228-related AEs by TAK-228 Dosing Schedule

Patients, *n* (%)	QD; *N* = 26	QDx3d QW; *N* = 13	Total; *N* = 39
Any drug-related grade ≥3 AE	8 (31)	10 (77)	18 (46)
Most common drug-related AEs			
Thrombocytopenia	2 (8)	4 (31)	6 (15)
Fatigue	1 (4)	3 (23)	4 (10)
Neutropenia	0	2 (15)	2 (5)
Anemia	0	1 (8)	1 (3)
Aphthous stomatitis	1 (4)	0	1 (3)
Asthenia	0	1 (8)	1 (3)
Hypocalcemia	1 (4)	0	1 (3)
Hypophosphatemia	1 (4)	0	1 (3)
Hypoxia	0	1 (8)	1 (3)
Lymphopenia	1 (4)	0	1 (3)
Mucosal inflammation	0	1 (8)	1 (3)
Pain	0	1 (8)	1 (3)
Pruritis	0	1 (8)	1 (3)
Rash erythematous	0	1 (8)	1 (3)
Stomatitis	1 (4)	0	1 (3)
Urticaria	1 (4)	0	1 (3)
Visual acuity reduced	1 (4)	0	1 (3)
White blood cell count decreased	0	1 (8)	1 (3)

AE, adverse event; QD, once daily; QDx3d QW, QD for 3 days on and 4 days off each week.

(46% each) (Supporting Information Table I); 77% of patients had any-cause grade ≥3 AEs and the most common AEs were thrombocytopenia (31%), fatigue (23%) and neutropenia (15%) (Supporting Information Table II). SAEs were reported in 31% of patients; 8% of patients had an SAE considered TAK-228-related. No on-study deaths due to AEs were reported on this dosing schedule.

Two AEs of interest were hyperglycemia and rash. Nine patients on the QD dosing schedule and six patients on the QDx3d QW dosing schedule reported hyperglycemia. All incidences of hyperglycemia were grade 1 or 2 in severity with no associated interruptions, reductions, or discontinuations of TAK-228 dosing. Four and three patients on the QD and QDx3d QW dosing schedules, respectively, reported rash—all related to study drug. One patient experienced a grade 3 erythematous rash (along with another grade 3 DLT event) and one patient had a grade 1 pruritic rash that resulted in discontinuation of study drug.

Pharmacokinetics

TAK-228 mean plasma concentration–time profiles on cycle 1, day 1 and cycle 2, day 1 for individual dose levels across dosing schedules are shown in Fig. 1; TAK-228 PK parameters are summarized in Supporting Information Table III. TAK-228 exhibited fast oral absorption with median time to maximum observed plasma concentration in the range of 1–2 hr. Plasma exposures of TAK-228 generally increased with dose from 2 to 7 mg QD and 9–12 mg QDx3d QW (Supporting Information Fig. 1). TAK-228 had a mean plasma elimination half-life of ~6 to 8 hr and did not accumulate in plasma with repeat dosing.

The PK profiles of TAK-228 were consistent across all dose levels during cycle 1 and 2 with the geometric mean CL/F of ~20 L h^{-1} and plasma accumulation ratio of ~1, indicating that there were no time-dependent changes in the PK of TAK-228. The dose-proportionality analysis on cycle 1 day 1 indicated that TAK-228 exposures generally increased in a dose-dependent manner, with the slope of the dose versus $AUC_{0\text{-inf}}$ curve of 1.03 (95% confidence interval [CI] 0.45–1.63) (Supporting Information Fig. 1).

Treatment response

Overall, there were 33 response-evaluable patients: 26 patients with MM, three with NHL, and four with WM. Six patients (five MM and one NHL) discontinued prior to the first response assessment and did not complete an efficacy assessment during the end of treatment visit. Three patients had an objective response.

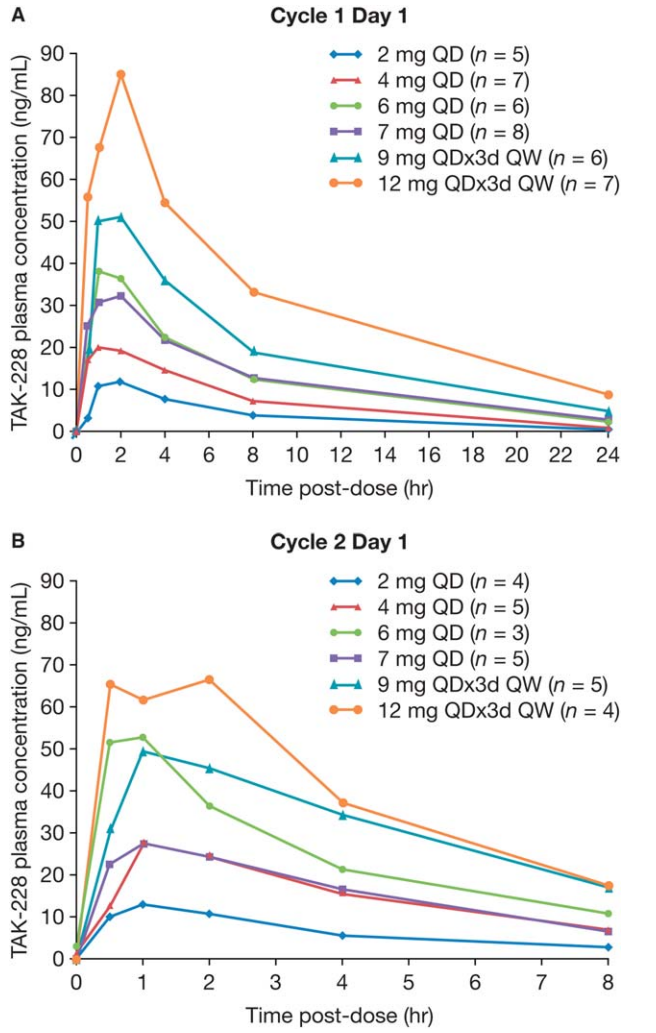

Figure 1. Mean TAK-228 plasma-concentration–time profile by dose level at (A) cycle 1 day 1 and (B) cycle 2 day 1.

One patient with MM achieved minimal objective response with duration of 66 days. The patient received TAK-228 at 7 mg QD through day 22, but was not dosed from day 23 to 29 due to an event of grade 3 thrombocytopenia. The patient resumed dosing on day 30 at 5 mg QD and continued to receive 5 mg QD through day 122. One patient with WM achieved partial response on day 169 that persisted until day 420 (duration of 252 days). This patient was on the 9-mg QDx3d QW dosing schedule and continued treatment through day 413. The third patient, also with WM, had a minor response on day 111 that continued until day 251 (duration of 141 days). This patient was on the 9-mg QDx3d QW dosing schedule up to day 167 but, from day 169 to 248, was on the 7-mg QDx3d QW dosing schedule due to an event of grade 3 generalized pain.

Two patients with MM had stable disease (SD) lasting ≥6 months, 12 had SD lasting <6 months and 11 patients had progressive disease. One patient with NHL had SD lasting ≥6 months, one had SD <6 months, and one had progressive disease. Two patients with WM achieved SD lasting ≥6 months and <6 months, respectively.

Discussion

This was the first clinical study of TAK-228 in hematologic malignancies, based on a preclinical rationale demonstrating activity in cell lines and xenografts [32,33] to characterize the safety profile and

assess preliminary evidence of antitumor activity in patients with MM, NHL, or WM. The MTD of TAK-228 was established for two different dosing schedules—4 mg QD and 9 mg QDx3d QW.

In the first phase I, dose-finding study of single-agent TAK-228 in patients with advanced solid malignancies (NCT01058707; INK128-001) [39], the MTD was slightly higher—6 mg QD and 12 mg QDx3d QW. One quarter (two of eight) of the DLT-evaluable patients in the 6-mg QD group had cycle 1 DLTs (grade 3 asthenia and maculo-papular rash) compared to half (three of six) of the patients in the present study (grade 2 increased blood creatinine, grade 3 fatigue, and grade 2 nausea and vomiting). In the 12-mg QDx3d QW group of the INK128-001 study, two of six patients reported cycle 1 DLTs of grade 3 stomatitis and grade 3 asthenia, dehydration, and mucosal inflammation. In the present study, cycle 1 DLTs in the 9-mg QDx3d QW group were grade 3 erythematous rash and fatigue in one of six patients. In a phase I study investigating TAK-228 in combination with paclitaxel in solid malignancies [40] (NCT01351350; INK128-003), the MTD was 10 mg QDx3d QW with two of nine patients reporting cycle 1 DLTs of grade 3 fatigue and macular rash. The incidence of fatigue was more frequent at higher doses of TAK-228. The incidence and severity of rash did not increase in a dose-proportional manner and were similar to rash observed with other PI3K/AKT/mTOR inhibitors [41,42].

Overall, inhibition of TORC1/2 in both MM and B-cell malignancies by the doses of TAK-228 shown here has an acceptable safety profile, consistent with other agents that inhibit the PI3K/AKT/mTOR pathway [41,43]. Similar to the other phase I studies [39,40] of TAK-228, 46% of patients reported grade $\geq$3 drug-related toxicities; the most common AEs were thrombocytopenia and fatigue. In contrast to trials evaluating rapalogs [44], incidences of hyperglycemia, a known toxicity associated with mTOR inhibition [43], were only grade 1 or 2 in severity and hyperglycemia was not dose-limiting in the present study. Based on the clinical experience in TAK-228 trials, glucose levels in patients were closely monitored and, when treatment was needed, patients responded quickly to metformin.

The PK profile of TAK-228 was consistent with findings from INK128-001 and INK128-003 studies. TAK-228 exhibited fast oral absorption following administration with exposures generally increasing in a dose-dependent manner. Lack of accumulation in the plasma after repeat dosing due to the short elimination half-life supports both daily and intermittent administration schedules.

Because of technical issues with the assays, PD findings from this study were not reported. Prior PD results from a single-agent TAK-228 study demonstrated treatment-related reductions in TORC1/2 skin biomarkers (phosphorylated S6, 4EBP1, and PRAS40) [39], which supports the dual TORC1/2 inhibitory activity of TAK-228.

Nearly half of the patients in this study achieved stable disease (14 with MM, two with NHL, and two with WM). In addition to the observed partial response in one patient and minor/minimal response in two patients, these findings are in line with responses seen in studies investigating single-agent everolimus in patients with MM [45], NHL [46], and WM [31]. The preliminary antitumor activity of TAK-228 supports further research in these patient populations.

Previous *in vitro* work demonstrated that TAK-228 was more potent than rapamycin and had more cytotoxic activity as well as synergistic cytotoxic activity in combination with agents currently used to treat MM (e.g., melphalan, dexamethasone, and bortezomib) in various cell lines [12,31]. Hence, there may be potential benefits in combination therapies with bortezomib and other agents for the treatment of MM, which warrant further investigation. Furthermore, a recent study investigating a pan-class I PI3K inhibitor showed impediment of cellular trafficking and survival in MM as well as WM cells [47], suggesting another potential combinatorial agent.

In the present study, TAK-228 as a single agent was well tolerated and exhibited preliminary therapeutic activity in hematologic malignancies. Furthermore, these findings support the possibility of combining TAK-228 with a mechanistically distinct agent to enhance antitumor activity by disrupting aberrant signals of the PI3K/AKT/mTOR pathway at multiple sites.

Acknowledgments

The authors thank all the study participants and their families, as well as the investigators, site staff, and members of the Multiple Myeloma Research Consortium involved in the study. The authors acknowledge the writing support of Dawn L. Lee of FireKite (an Ashfield company, part of UDG Healthcare plc) in the development of this manuscript.

Author Contributions

Study conception and design—IMG, RN, JLW. Collection and assembly of data—IMG, DSS, RN, CGP, FZ. Data analysis and interpretation—IMG, DSS, RV, JGB, PGR, RN, CGP, FZ. Provision of study materials or patients—IMG, RV, PGR, JGB, FZ, JLW. All authors (IMG, DSS, RV, JGB, PGR, RN, CGP, FZ, and JLW) contributed to drafting or revising the manuscript, and reviewed and approved the final version of the manuscript.

Author Disclosures

IMG: Advisory Board or consultant role—Millennium Pharmaceuticals, Inc., Novartis, Celgene, BMS and Onyx. DSS: Speakers bureau and consultant/advisory role—Celgene, Millennium Pharmaceuticals, Inc., Onyx. RV: Research funding and consultant/advisory role—Millennium Pharmaceuticals, Inc. JGB: Received grant and served in an independent review committee for Millennium Pharmaceuticals, Inc. PGR: Consultant/advisory role—Millennium Pharmaceuticals, Inc. RN, CGP, FZ: Employment by Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited. JLW: None.

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