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Phase I study of the investigational oral mTORC1/2 inhibitor sapanisertib (TAK-228): tolerability and food effects of a milled formulation in patients with advanced solid tumours

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ABSTRACT

Background Sapanisertib (TAK-228) is an investigational, orally available, potent and highly selective mTORC1/2 inhibitor demonstrating promise in numerous malignancies. This phase I study (NCT02412722) evaluated the safety, tolerability, pharmacokinetics and antitumour activity of single-agent TAK-228 (milled capsules), administered daily (QD) or weekly (QW) and in combination with paclitaxel in patients with advanced solid tumours. Pharmacokinetic comparisons of milled versus unmilled TAK-228 and the impact of food were also investigated.

Methods Patients were enrolled to receive: TAK-228 QD, TAK-228 3 days/week plus paclitaxel 80 mg/m^2 days 1, 8, 15 (TAK-228+P) or TAK-228 QW (all 28-day cycles); starting TAK-228 doses were 4, 6 and 20 mg, respectively.

Results Sixty-one adults were enrolled. Maximum tolerated doses for milled TAK-228 were 3 mg (TAK-228 QD), 6 mg (TAK-228+P) and 30 mg (TAK-228 QW). Most patients reported ≥1 adverse event (AE); there were no meaningful differences in drug-related AEs across regimens or doses. Three on-study deaths occurred, all considered unrelated to study drugs. TAK-228 pharmacokinetics did not differ between unmilled/milled capsules or with/without paclitaxel. However, TAK-228 C_{max} decreased by ~40% in fed versus fasted patients. Objective response rates were 12% (TAK-228 QD), 18% (TAK-228+P) and 0% (TAK-228 QW). One patient receiving TAK-228+P had a complete response; three patients receiving TAK-228+P and two patients receiving TAK-228 QD had partial responses.

Conclusions Milled TAK-228 was well tolerated with signs of antitumour activity; administration did not reduce overall exposure (area under the plasma concentration–time curve) but reduced C_{max}, which is expected when dosed in the fed state. These promising findings warrant further investigation.

Trial registration number NCT02412722.

Key questions

What is already known about this subject?

- TAK-228 is an investigational, orally available and highly selective ATP-competitive inhibitor of both mTORC1 and mTORC2.
- TAK-228 has displayed evidence of anticancer activity when given to patients with non-haematological malignancies.
- The maximum tolerated dose (MTD) and recommended phase II dose for the unmilled active pharmaceutical ingredient TAK-228 when taken as capsules after a light meal has been determined for several dosing regimens and in combination with other anticancer agents.

What does this study add?

- New MTDs were determined for capsules containing milled TAK-228 given once daily, once weekly or in combination with paclitaxel (at lower doses than in previous studies of unmilled TAK-228).
- The TAK-228 pharmacokinetic profile did not differ between the milled and unmilled capsules or with/without paclitaxel. However, C_{max} was ~40% lower when TAK-228 was given to fed patients compared with those who had fasted.
- Antitumour activity was seen in patients receiving the milled capsules, encouraging further investigation of TAK-228.

How might this impact on clinical practice?

- In the longer term, these findings may influence dosing considerations for TAK-228, both alone and in combination with paclitaxel or other drugs, in further investigational studies.

INTRODUCTION

The mammalian (or 'mechanistic') target of rapamycin (mTOR) is a serine/threonine kinase activated by external stimuli via the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinases signalling pathways.[1] mTOR forms two distinct multisubunit complexes, mTORC1 and mTORC2, to regulate protein expression,

cell growth, survival, actin cytoskeletal dynamics and metabolism. Aberrant mTOR signalling is common in cancer and contributes to solid tumour development and progression.[2 3]

First-generation mTOR inhibitors, such as sirolimus (rapamycin), everolimus and temsirolimus, are allosteric inhibitors that primarily inhibit mTORC1.[4] However, rapamycin and its analogues may inadvertently upregulate protein kinase B (Akt) activity and paradoxical hyperactive signalling, which can lead to enhanced survival of tumour cells and chemoresistance.[4 5] Furthermore, evidence suggests that a temsirolimus-resistant phenotype may be mediated through the activation of signal transduction pathways via mTORC2, not mTORC1[6]; hence, new agents capable of dual mTORC1/2 inhibition may be preferable to existing drugs that target only mTORC1.

TAK-228 is an investigational, orally available and highly selective ATP-competitive inhibitor of both mTORC1 and mTORC2.[7] TAK-228 is being developed both as monotherapy and in combination with other agents for the treatment of advanced solid tumours and has demonstrated an acceptable safety profile and preliminary therapeutic activity in two phase I trials.[7 8] Both studies used capsules of TAK-228 comprising unmilled active pharmaceutical ingredient, which patients took with a light meal. Subsequent improvements to the TAK-228 manufacturing process included a physical milling step following granulation to improve particle size distribution and enable automated capsule filling for scaling-up manufacturing.[9 10] Initial pharmacokinetic (PK) simulations using GastroPlus (Simulations Plus, Lancaster, California, USA) suggested that milled and unmilled TAK-228 capsules dosed up to 6 mg under fasting conditions would result in similar maximum plasma concentrations (C_{max}) and exposure as measured by the area under the concentration–time curve (AUC). However, under fed conditions, milled TAK-228 was predicted to have 65% higher C_{max} and earlier time of maximum plasma concentration (T_{max}) compared with unmilled TAK-228, with no meaningful differences in the AUC (data on file). In order to enable the integration of the new milled capsule formation of TAK-228 into the clinical development programme and to understand the safety data in the context of the historic PK and safety data for TAK-228, an evaluation of the PK of TAK-228 comparing the different dosing conditions and formulations was important.

This phase I study (NCT02412722; MLN0128-1004) evaluated the safety, tolerability, PK and preliminary efficacy of milled TAK-228 capsule formulation administered as a single agent or in combination with paclitaxel under fasted dosing conditions in adult patients with advanced non-haematological malignancies. The study also evaluated the effect of food (fasted or with a high-fat meal) and manufacturing process (ie, milled vs unmilled TAK-228) on the PK of TAK-228 capsules.

METHODS

Study design

This open-label, phase I study was conducted in adult patients with advanced non-haematological malignancies across four sites in the USA. The primary objectives were to evaluate the safety and tolerability of milled TAK-228 as a single agent and in combination with paclitaxel, to characterise the effect of dosing condition (fasted or with a high-fat meal) on the PK of TAK-228 and to characterise the PK of milled TAK-228 taken under fasted conditions ~24 hours after paclitaxel infusion. The secondary objectives were to characterise the PK of milled versus unmilled TAK-228 (fasted conditions) and to evaluate the preliminary efficacy of milled TAK-228 (as a single agent and in combination with paclitaxel).

Patients were assigned to one of three treatment arms (online supplementary figure 1) at the discretion of the investigator and based on cohort availability: (1) milled TAK-228 once daily (QD) on days 1–28 (starting dose 4 mg); (2) milled TAK-228 QD for 3 days per week on days 2–4, 9–11, 16–18 and 23–25 (starting dose 6 mg) and paclitaxel (P) intravenous infusion on days 1, 8 and 15 dosed ~24 hours prior to TAK-228; and (3) milled TAK-228 administered once weekly (QW) on days 1, 8, 15 and 22 (starting dose 20 mg). Patients enrolled in the TAK-228 QD arm also participated in a PK run-in period conducted ≤14 days prior to cycle 1 day 1 (online supplementary figure 1). On three separate visits separated by ≥48 hours during the PK run-in period, patients received unmilled TAK-228 4 mg on an empty stomach (visit 1), milled TAK-228 4 mg after a high-fat breakfast (visit 3) and milled TAK-228 4 mg on an empty stomach (visit 5). Collection of PK samples at 24 hours post-dose timepoints were conducted during visits 2, 4 and 6. Patients in all treatment arms received TAK-228 in repeated 28-day cycles until disease progression or unacceptable toxicity.

Starting doses of TAK-228 were based on previously observed maximum tolerated doses (MTDs)[7 11] and the expectation that milled TAK-228 formulations would increase drug exposure. Enrolment into further dosing cohorts was dependent on the observation of dose-limiting toxicities (DLTs) according to dose escalation rules outlined in online supplementary figure 2. DLTs were defined as adverse events (AEs) associated with the study drug serious enough to prevent further increases in the dosage. Details of specific DLTs are included in the appendix. The MTD was defined as the dose of TAK-228 resulting in ≤1 DLT in cycle 1 in all treatment arms.

A minimum of 16 patients completing the protocol in the single-agent TAK-228 QD treatment arm was selected as adequate to enable precision in estimating geometric mean ratios. Sample sizes for TAK-228 QW and TAK 228+P arms were based on clinical considerations.

Patients

The study enrolled patients aged ≥18 years with advanced non-haematological malignancy who had failed on, or

were not eligible for, standard-of-care therapy. Key inclusion criteria included adequate organ function, Eastern Cooperative Oncology Group performance status of 0–1 and no more than four prior lines of cytotoxic chemotherapy. Patients were excluded if they had received diagnosis of a primary, untreated metastatic brain tumour, history of leptomeningeal disease or spinal cord compression. Further exclusion criteria included failure to recover from reversible side effects of anticancer therapy, poorly managed diabetes mellitus, significant cardiovascular or pulmonary disease within the 6 months prior to study start and manifestations of gastrointestinal malabsorption. Patients participating in the TAK-228 QD PK run-in were excluded if they had used proton pump inhibitors within 5 days or H_2-receptor antagonists within 24 hours of the first PK run-in dose.

Informed written consent was obtained from all patients.

Assessments

Serial blood samples for PK analysis were taken 30 min prior to administration of TAK-228 and 30 min, 1, 2, 3, 4, 6, 8 and 24 hours after administration of TAK-228 on visits 1, 3 and 5 of the QD run-in period, day 2 of cycle 1 in the TAK-228+P arm (first day of TAK-228 treatment) and day 1 of cycle 1 of the TAK-228 QW arm. Blood samples were analysed using liquid chromatography with tandem mass spectrometry (Frontage Laboratories, Inc.), validated over the concentration range 1–1000 ng/mL.

AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 and coded to standardised terms using the Medical Dictionary for Regulatory Activities version 19.0. Serious AEs (SAEs) and AEs were recorded from the first dose of the study drug through 30 days after the last dose administration. The potential relatedness of an AE to the study drug was determined by the investigator.

Disease status and best overall response, objective response rate (ORR; complete response (CR)+partial response (PR)) and clinical benefit rates (CR+PR+stable disease (SD) and CR+PR+SD for ≥6 months) were assessed in accordance with the Response Evaluation Criteria in Solid Tumours (version 1.1)[12] guidelines using contrast CT or MRI of the chest, abdomen and pelvis. Bone scans could be used in place of CT or MRI in patients with bone metastases. Baseline scans were taken within 4 weeks of the first administration of study drug, and subsequent assessments were carried out on day 1 of cycle 3 and every two cycles thereafter until the end of the study or treatment discontinuation. The same imaging modality was used for each patient throughout the study.

Statistical methods

The PK population, which was used for all PK analyses, included all patients with protocol-specified dosing, conditions and PK data required to reliably estimate PK parameters. The DLT-evaluable population comprised all patients who received ≥75% of planned TAK-228 doses in cycle 1 or who stopped taking TAK-228 before receiving 75% of the planned TAK-228 doses because of a drug-related AE.

Plasma concentration and calculated PK parameters were summarised by descriptive statistics. For plasma PK parameters, linear and semilogarithmic plots of the mean plasma concentration versus scheduled sampling time, and individual plasma concentration versus actual sampling time were provided with descriptive statistics. For data collected during the TAK-228 QD PK run-in, analysis of variance was performed with log-transformed C_{max} and AUC as dependent variables, treatment as a fixed effect and patient as a random effect. In addition, geometric mean ratios of fed versus fasted dosing conditions, and milled versus unmilled TAK-228 capsules, were calculated with 90% CI calculated for the difference in the least square means on the natural log-transformed PK parameters.

RESULTS

From 26 March 2015 to 30 June 2016, 61 patients (age range, 28–88 years) with non-haematological malignancies (most commonly stage IV) were enrolled and received at least one dose of study drug (TAK-228 QD, n=19; TAK-228+P, n=22; TAK-228 QW, n=20) (table 1). The most commonly diagnosed cancers were uterine cancer (n=7), colon cancer (n=6) and ovarian cancer (n=5). At data cut-off, 56 patients had discontinued (26 due to progressive disease, 8 due to AEs, 7 withdrew, 1 was lost to follow-up and 14 for other reasons), with five ongoing.

DLTs and MTD determination

In the TAK-228 QD arm, 3/6 DLT-evaluable patients receiving TAK-228 4 mg experienced at least one DLT (grade 3 fatigue; grade 3 fatigue, macular rash and decreased appetite; and grade 2 and 3 rash). Of the 10 patients enrolled to receive TAK-228 3 mg, one patient experienced a DLT (grade 3 thrombocytopaenia). Thus, the MTD and recommended phase II dose (RP2D) for single-agent QD milled TAK-228 was established as 3 mg. In the TAK-228+P arm, one of seven patients receiving TAK-228 6 mg experienced three DLTs (grades 2 and 3 fatigue and grade 3 dehydration), and nine SAEs in four patients were considered related to treatment; on expansion of the 6 mg cohort to 12 patients, no additional DLTs were observed within cycle 1, but five of these 12 patients required a reduction in TAK-228 in subsequent cycles. No DLTs were observed in a separate cohort (n=7, 6 DLT evaluable) given TAK-228 4 mg plus paclitaxel. Thus, when given in combination with paclitaxel 80 mg/m^2, TAK-228 4 mg was identified as the RP2D, and TAK-228 6 mg was determined as the MTD. In the TAK-228 QW arm, none of the six patients receiving 20 mg experienced a DLT. A subsequent cohort initially consisting of six DLT-evaluable patients was given TAK-228 30 mg QW, with none experiencing DLTs; on cohort expansion to 12 patients, 1

Table 1 Patient demographics and baseline characteristics

	TAK-228 QD			TAK-228+P			TAK-228 QW		
	3 mg (n=11)	4 mg (n=8)	Total (n=19)	4 mg (n=7)	6 mg (n=15)	Total (n=22)	20 mg (n=7)	30 mg (n=13)	Total (n=20)
Median age, years (range)	65 (35–88)	61 (46–73)	62 (35–88)	52 (49–68)	65 (34–81)	63 (34–81)	61 (28–76)	66 (39–79)	64 (28–79)
Female, n (%)	9 (82)	6 (75)	15 (79)	5 (71)	11 (73)	16 (73)	3 (43)	10 (77)	13 (65)
Race, n (%)									
White	9 (82)	7 (88)	16 (84)	6 (86)	11 (73)	17 (77)	5 (71)	11 (85)	16 (80)
Black or African-American	1 (9)	1 (13)	2 (11)	1 (14)	2 (13)	3 (14)	1 (14)	1 (8)	2 (10)
Other	1 (9)	0	1 (5)	0	2 (13)	2 (9)	1 (14)	1 (8)	2 (10)
ECOG PS									
0	5 (46)	3 (38)	8 (42)	2 (29)	7 (47)	9 (41)	3 (43)	4 (31)	7 (35)
1	6 (55)	5 (63)	11 (58)	5 (71)	8 (53)	13 (59)	4 (57)	9 (69)	13 (65)
Primary diagnosis, n (%)									
Anal	–	–	–	1 (14)	0	1 (5)	–	–	–
Bile duct	–	–	–	1 (14)	1 (7)	2 (9)	1 (14)	0	1 (5)
Bone	–	–	–	–	–	–	0	1 (8)	1 (5)
Breast	0	1 (13)	1 (5)	1 (14)	1 (7)	2 (9)	0	1 (8)	1 (5)
Colon	1 (9)	3 (38)	4 (21)	–	–	–	1 (14)	1 (8)	2 (10)
Endometrium	1 (9)	0	1 (5)	–	–	–	0	1 (8)	1 (5)
Fallopian tube	2 (18)	0	2 (11)	–	–	–	–	–	–
Gall bladder	–	–	–	–	–	–	1 (14)	0	1 (5)
Head/neck	–	–	–	1 (14)	0	1 (5)	1 (14)	2 (15)	3 (15)
Kidney	1 (9)	0	1 (5)	–	–	–	–	–	–
Lung	–	–	–	0	2 (13)	2 (9)	1 (14)	1 (8)	2 (10)
Neuroendocrine	0	1 (13)	1 (5)	0	1 (7)	1 (5)	–	–	–
Ovary	1 (9)	0	1 (5)	0	1 (7)	1 (5)	0	3 (23)	3 (15)
Pancreas	1 (9)	0	1 (5)	0	1 (7)	1 (5)	0	1 (8)	1 (5)
Peritoneum	–	–	–	0	1 (7)	1 (5)	–	–	–
Prostate	–	–	–	0	1 (7)	1 (5)	–	–	–
Rectal	1 (9)	0	1 (5)	–	–	–	1 (14)	0	1 (5)
Soft-tissue sarcoma	1 (9)	1 (13)	2 (11)	2 (29)	0	2 (9)	–	–	–
Stomach	0	1 (13)	1 (5)	–	–	–	–	–	–
Thymus	–	–	–	–	–	–	1 (14)	0	1 (5)
Unknown	1 (9)	0	1 (5)	0	1 (7)	1 (5)	–	–	–

Continued

Table 1 Continued

	TAK-228 QD			TAK-228+P			TAK-228 QW		
	3 mg (n=11)	4 mg (n=8)	Total (n=19)	4 mg (n=7)	6 mg (n=15)	Total (n=22)	20 mg (n=7)	30 mg (n=13)	Total (n=20)
Urinary bladder	–	–	–	0	1 (7)	1 (5)	–	–	–
Uterus	1 (9)	1 (12)	2 (11)	1 (14)	4 (27)	5 (23)	–	–	–
Missing	–	–	–	–	–	–	0	2 (15)	2 (10)
Disease stage at initial diagnosis, n (%)									
I	1 (9)	0	1 (5)	2 (29)	0	2 (9)	1 (14)	2 (15)	3 (15)
II	1 (9)	2 (25)	3 (16)	1 (14)	1 (7)	2 (9)	1 (14)	4 (31)	5 (25)
III	3 (27)	1 (13)	4 (21)	0	5 (33)	5 (23)	0	4 (31)	4 (20)
IV	5 (46)	3 (38)	8 (42)	2 (29)	8 (53)	10 (46)	5 (71)	2 (15)	7 (35)
Missing	1 (9)	2 (25)	3 (16)	2 (29)	1 (7)	3 (14)	0	1 (8)	1 (5)

–, not available/not applicable; ECOG PS, Eastern Cooperative Oncology Group performance status; QD, once daily; QW, once weekly; TAK-228+P, TAK-228 QD 3 days/week+paclitaxel 80 mg/m^2 on days 1, 8, 15.

patient had a DLT (grade 3 adverse drug reaction). Both the MTD and RP2D for the TAK-228 QW were determined as 30 mg.

Safety and tolerability

Of 19 patients participating in the TAK-228 QD PK run-in, 17 participated in the QD treatment phase and were included in the safety analyses (n=59). With TAK-228 QD, patients received a median of three cycles (range, 1–13) and a mean (standard deviation) of 4.7 (4.1) cycles, with longer exposure for 3 versus 4 mg (4 vs 1.5 cycles; 15.0 vs 7.4 weeks). With TAK-228+P, patients received a median of two cycles (range, 1–9) and a mean (standard deviation) of 3.2 (2.2) TAK-228 cycles; median duration of exposure was similar with TAK-228 4 and 6 mg (7.6 and 7.7 weeks, respectively), as was median cumulative paclitaxel dose (478.9 and 479.1 mg, respectively). With TAK-228 QW, patients received a median of two cycles (range 1–10) and a mean (standard deviation) of 2.7 (2.1) cycles; median duration of exposure was 7.1 weeks with both 20 mg and 30 mg TAK-228.

AEs were experienced by 100%, 96% and 95% of patients in the TAK-228 QD, TAK-228+P and TAK-228 QW arms, respectively; 94%, 96% and 80% were considered drug-related (table 2). The most common drug-related AEs of any grade were fatigue (59%), pruritus (41%), decreased appetite, diarrhoea and nausea (29% each) with TAK-229 QD; diarrhoea (64%), decreased appetite, fatigue, nausea (41% each) and stomatitis (36%) with TAK-228+P; and fatigue, nausea, vomiting (45% each) and diarrhoea (30%) with TAK-228 QW. The most common grade ≥3 AEs were macular rash, fatigue, hyponatraemia and hyperglycaemia (12% each) with TAK-228 QD; neutropaenia, fatigue (18% each), abdominal pain and hypophosphataemia (14% each) with TAK-228+P; and hyperglycaemia (15%), abdominal pain, anaemia, hypercalcaemia, hypotension, nausea and vomiting (10% each) with TAK-228 QW (table 2).

AEs of interest for this study (hyperglycaemia, rash, renal insufficiency, mucosal inflammation and asthaenic conditions) were observed in ≥10% of patients in all treatment arms. Asthaenic conditions were the most common AE of interest, occurring in 59%, 55% and 60% of patients treated with TAK-228 QD, TAK-228+P and TAK-228 QW, respectively; these were mostly cases of fatigue.

SAEs were reported in 29% of patients treated with TAK-228 QD, 41% of patients treated with TAK-228+P and 50% of patients treated with TAK-228 QW (table 2). One patient receiving TAK-228 4 mg QD, three patients receiving TAK-228+P 6 mg and three patients receiving TAK-228 30 mg QW discontinued TAK-228 treatment due to AEs, and four patients discontinued paclitaxel. Three deaths occurred during the study, one in each treatment arm; none were considered related to study drugs.

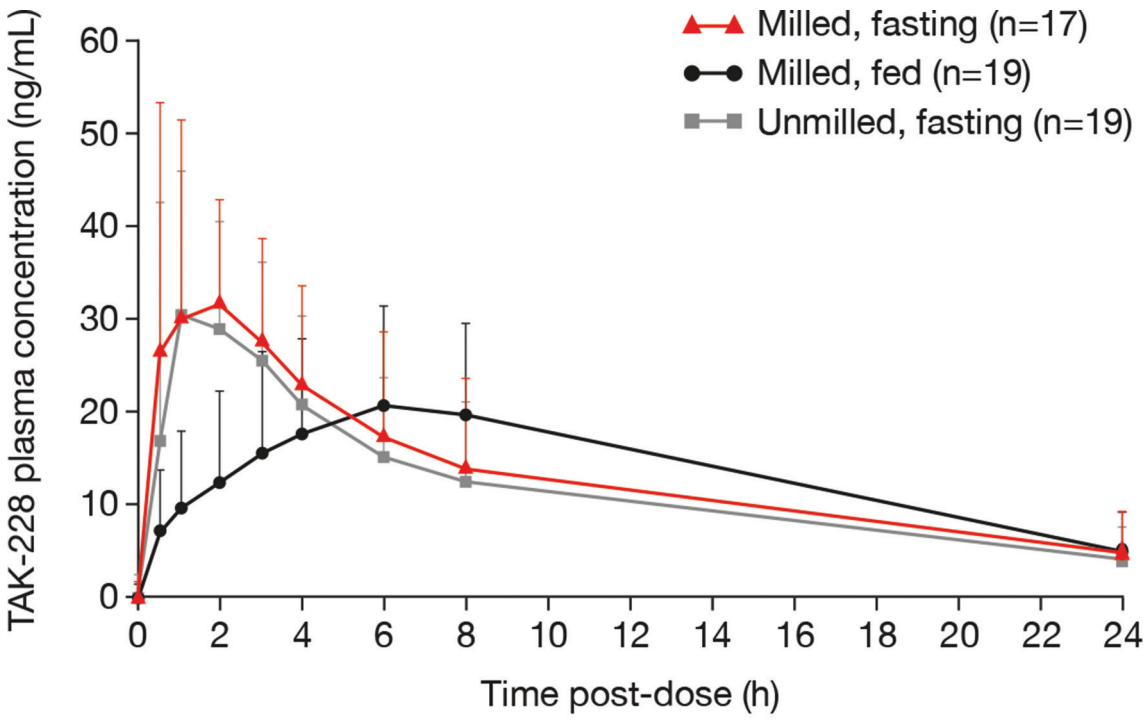
PK profile

There were no appreciable differences in the concentration–time profile or PK parameters of TAK-228

Table 2 Safety profile of TAK-228 during the dosing phase, including all cause grade ≥3 AEs occurring in more than one patient and the most common any-grade drug-related AEs occurring in ≥15% of patients

	TAK-228 QD			TAK-228+P			TAK-228 QW		
	3 mg (n=11)	4 mg (n=6)	Total (n=17)	4 mg (n=7)	6 mg (n=15)	Total (n=22)	20 mg (n=7)	30 mg (n=13)	Total (n=20)
On-study deaths, n (%)	0	1 (17)	1 (6)	0	1 (7)	1 (5)	0	1 (8)	1 (5)
AE resulting in TAK-228 discontinuation, n (%)	0	1 (17)	1 (6)	0	3 (20)	3 (14)	0	3 (23)	3 (15)
AE resulting in paclitaxel discontinuation, n (%)	–	–	–	0	4 (27)	4 (18)	–	–	–
Drug-related SAE, n (%)	0	0	0	1 (14)	4 (27)	5 (23)	0	2 (15)	2 (10)
SAE, n (%)	3 (27)	2 (33)	5 (29)	2 (29)	7 (47)	9 (41)	4 (57)	6 (46)	10 (50)
Drug-related grade ≥3 AE, n (%)	5 (46)	5 (83)	10 (59)	2 (29)	11 (73)	13 (59)	2 (29)	4 (31)	6 (30)
Grade ≥3 AE, n (%)	6 (55)	6 (100)	12 (71)	4 (57)	11 (73)	15 (68)	5 (71)	7 (54)	12 (60)
Abdominal pain	1 (9)	0	1 (6)	2 (29)	1 (7)	3 (14)	1 (14)	1 (8)	2 (10)
Anaemia	0	0	0	0	2 (13)	2 (9)	1 (14)	1 (8)	2 (10)
Asthaenia	–	–	–	1 (14)	1 (7)	2 (9)	–	–	–
Blood phosphorus decreased	1 (9)	0	1 (6)	0	2 (13)	2 (9)	–	–	–
Dehydration	–	–	–	0	2 (13)	2 (9)	–	–	–
Diarrhoea	1 (9)	0	1 (6)	0	2 (13)	2 (9)	1 (14)	–	1 (5)
Fatigue	0	2 (33)	2 (12)	0	4 (27)	4 (18)	–	–	–
Hypercalcaemia	–	–	–	–	–	–	0	2 (15)	2 (10)
Hyperglycaemia	1 (9)	1 (17)	2 (12)	0	1 (7)	1 (5)	0	3 (23)	3 (15)
Hyponatraemia	1 (9)	1 (17)	2 (12)	–	–	–	–	–	–
Hypophosphataemia	–	–	–	0	3 (20)	3 (14)	0	1 (8)	1 (5)
Hypotension	–	–	–	–	–	–	2 (29)	0	2 (10)
Nausea	–	–	–	0	2 (13)	2 (9)	1 (14)	1 (8)	2 (10)
Neutropaenia	–	–	–	0	4 (27)	4 (18)	–	–	–
Rash macular	1 (9)	1 (17)	2 (12)	–	–	–	–	–	–
Vomiting	–	–	–	0	1 (7)	1 (5)	1 (14)	1 (8)	2 (10)
Drug-related AE, n (%)	10 (91)	6 (100)	16 (94)	7 (100)	14 (93)	21 (96)	5 (71)	11 (85)	16 (80)
Abdominal pain	2 (18)	1 (17)	3 (18)	–	–	–	–	–	–
Alopecia	–	–	–	2 (29)	2 (13)	4 (18)	–	–	–
Asthaenia	–	–	–	1 (14)	4 (27)	5 (23)	1 (14)	0	1 (5)
Decreased appetite	3 (27)	2 (33)	5 (29)	3 (43)	6 (40)	9 (41)	1 (14)	2 (15)	3 (15)
Dehydration	–	–	–	0	4 (27)	4 (18)	1 (14)	0	1 (5)
Diarrhoea	2 (18)	3 (50)	5 (29)	5 (71)	9 (60)	14 (64)	4 (57)	2 (15)	6 (30)
Dry mouth	–	–	–	2 (29)	2 (13)	4 (18)	0	1 (8)	1 (5)
Fatigue	6 (55)	4 (67)	10 (59)	1 (14)	8 (53)	9 (41)	3 (43)	6 (46)	9 (45)
Hyperglycaemia	2 (18)	1 (17)	3 (18)	1 (14)	3 (20)	4 (18)	0	3 (23)	3 (15)
Nausea	4 (36)	1 (17)	5 (29)	4 (57)	5 (33)	9 (41)	4 (57)	5 (39)	9 (45)
Neuropathy peripheral	–	–	–	3 (43)	2 (13)	5 (23)	–	–	–
Neutropaenia	–	–	–	0	4 (27)	4 (18)	–	–	–
Pruritus	4 (36)	3 (50)	7 (41)	–	–	–	0	1 (8)	1 (5)
Rash	1 (9)	2 (33)	3 (18)	1 (14)	0	1 (5)	–	–	–
Rash (maculopapular)	4 (36)	0	4 (24)	2 (29)	0	2 (9)	0	2 (15)	2 (10)
Stomatitis	1 (9)	2 (33)	3 (18)	4 (57)	4 (27)	8 (36)	0	2 (15)	2 (10)
Vomiting	1 (9)	1 (17)	2 (12)	2 (29)	5 (33)	7 (32)	2 (29)	7 (54)	9 (45)

–, not applicable; AE, adverse event; QD, once daily; QW, once weekly; SAE, serious adverse event; TAK-228+P, TAK-228 QD 3 days/week+paclitaxel 80 mg/m^2 on days 1, 8, 15.



	Comparison of formulation			Evaluation of dosing condition (milled TAK-228)		
PK parameters	Milled	Unmilled	Ratio* (90% CI)	Fed	Fasted	Ratio* (90% CI)
C_{max},* ng/mL (%CV)	36.4 (50.6) n=17	34.0 (48.1) n=19	1.07 (0.78, 1.47)	21.6 (37.4) n=19	36.4 (50.6) n=17	0.59 (0.46, 0.76)
AUC_{24hr},* hr•ng/mL (%CV)	316.5 (40.5) n=13	273.8 (40.5) n=14	1.16 (0.84, 1.60)	262.5 (47.1) n=16	316.5 (40.5) n=13	0.83 (0.62, 1.10)
AUC_{last},* hr•ng/mL (%CV)	229.2 (58.2) n=17	204.6 (57.3) n=19	1.12 (0.75, 1.68)	205.9 (59.8) n=19	229.2 (58.2) n=17	0.90 (0.59, 1.36)
AUC_{∞},* hr•ng/mL (%CV)	369.2 (50.0) n=13	400.8 (48.2) n=13	0.92 (0.67, 1.26)	382.8 (64.7) n=13	369.2 (50.0) n=13	1.04 (0.72, 1.49)
T_{max},† hr (range)	2.0 (0.5–3.1) n=17	1.7 (0.5–6.1) n=19	–	6.0 (1.0–8.0) n=19	2.0 (0.5–3.1) n=17	–
$t_{½}$,‡ hr (SD)	8.3 (2.5) n=13	12.8 (8.0) n=13	–	10.6 (7.2) n=13	8.3 (2.5) n=13	–
CL/F,* L/hr (%CV)	10.8 (47.0) n=13	10.0 (41.3) n=13	1.09 (0.79, 1.49)	10.5 (52.1) n=13	10.8 (47.0) n=13	0.96 (0.67, 1.39)
Vz/F,* L (%CV)	124.2 (29.4) n=13	153.4 (65.2) n=13	0.81 (0.61, 1.07)	132.8 (29.6) n=13	124.2 (29.4) n=13	1.07 (0.88, 1.30)

Figure 1 Plasma concentration–time profile, relative bioavailability and pharmacokinetic (PK) parameters of 4 mg TAK-228 in the single-agent QD PK run-in period, according to manufacturing process and dosing conditions. *Geometric mean; †Median; ‡Arithmetic mean. –, not available/not applicable; AUC, area under the plasma concentration–time curve; CL/F, apparent oral clearance; C_{max}, maximum plasma concentration; CV, coefficient of variation; $t_{½}$, plasma half-life; TAK-228+P, TAK-228 once daily 3 days/week+paclitaxel 80 mg/m^2 on days 1, 8 and 15; T_{max}, time of maximum plasma concentration; QW, once weekly; Vz/F, apparent terminal phase volume of distribution.

Table 3 Pharmacokinetic profile of TAK-228 given three times weekly in combination with paclitaxel (TAK-228+P) or as monotherapy once weekly (TAK-228 QW)

	TAK-228+P		TAK-228 QW	
Parameter	4 mg (n=7)	6 mg (n=15)	20 mg (n=7)	30 mg (n=13)
C_{max},* ng/mL (CV)	28.5 (64)	65.7 (41)	208.6 (17)	235.2 (43)
AUC_{8hr},* hour·ng/mL	124.1 (58)	243.9 (34)	–	–
AUC_{24hr},* hour·ng/mL (CV)	–	–	1238.1 (53)	1528.8 (44)
AUC_{∞},* hour·ng/mL (CV)	–	–	1326.2 (62)	1636.6 (45)
T_{max},† hour (range)	2.2 (0.9–5.6)	1.1 (0.5–3.0)	1.0 (0.9–2.3)	1.0 (0.5–8.3)
$t_{½}$,‡ hour (standard deviation)	–	–	6.1 (2.3)	6.0 (1.6)
CL/F,* L/hour (CV)	–	–	15.1 (46)	18.3 (63)
Vz/F,* L (CV)	–	–	125.1 (28)	154.9 (44)

*Geometric mean.
†Median.
‡Arithmetic mean.
AUC, area under the curve; C_{max}, maximum plasma concentration; CL/F, apparent oral clearance; CV, coefficient of variation; QW, once weekly; T_{max}, time of maximum plasma concentration; TAK-228+P, TAK-228 once daily 3 days/week+paclitaxel 80 mg/m^2 on days 1, 8 and 15; Vz/F, apparent terminal phase volume of distribution.

administered as unmilled versus milled TAK-228 4 mg capsules in a fasted state (figure 1). Geometric mean C_{max} (34.0 vs 36.4 ng/mL for unmilled vs milled TAK-228 capsules, respectively) and median T_{max} (1.7 vs 2.0 hours, respectively) were comparable between the two manufacturing processes. In contrast, there was a clinically significant reduction of ~40% in the geometric mean C_{max} of milled TAK-228 capsules administered following consumption of a high-fat breakfast compared with administration on an empty stomach (21.6 vs 36.4 ng/mL, respectively) (figure 1), but no appreciable change in the geometric mean AUC_{∞} of milled TAK-228 following a high-fat meal versus in a fasted state (382.8 vs 369.2 hour·ng/mL, respectively). The mean T_{max} of milled TAK-228 increased from 2.0 hours in the fasted state to 6.0 hours in the fed state (figure 1).

The PK of TAK-228 was consistent when administered as milled TAK-288 capsules on a QW schedule, with a dose-dependent increase in plasma exposure between the 20 mg and 30 mg doses (table 3). The plasma exposure of TAK-228 administered in combination with paclitaxel also increased in a dose-dependent manner between the 4 mg and 6 mg doses. PK parameters for TAK-228 in the combination arm (table 3) were consistent with PK for TAK-228 (4 mg, milled, fasted state) administered in the QD arm (figure 1), suggesting that administering paclitaxel 24 hours before TAK-228 had no clinically meaningful effects on TAK-228 PK.

Tumour response

With TAK-228 QD, two patients (one each with renal and uterine cancers (TAK-228 3 mg and 4 mg, respectively)) achieved a PR, equating an ORR of 12% (table 4). Seven patients had SD, four of whom maintained SD for ≥6 months: individual patients had SD duration of 208 days (TAK-228 3 mg; fallopian tube cancer), 220 days (TAK-228 3 mg; fallopian tube cancer), 253 days (TAK-228 3 mg; ovarian cancer) and 368 days (TAK-228 4 mg; colon cancer) (online supplementary figure 3). The overall clinical benefit rate (CR+PR + SD) was 52.9% (9/17 patients). Apart from a greater incidence of SD in patients receiving 3 mg TAK-228, there were no notable trends in efficacy across the different dose levels.

With TAK-228+P, one patient (breast cancer) achieved a CR and three achieved PRs (one each with urinary bladder and uterine cancers (both TAK-228 6 mg), one with soft-tissue sarcoma (TAK-228 4 mg)), resulting in an ORR of 18% (table 4). Seven patients achieved SD; one patient with ovarian cancer receiving TAK-228 6 mg plus paclitaxel maintained SD for ≥6 months (220 days) (online supplementary figure 3). The overall clinical benefit rate with TAK-228+P was 50% (11/22 patients): no notable trends were observed between the 4 mg and 6 mg TAK-228 doses.

No patient achieved an objective response with TAK-228 QW. Nine patients achieved SD; one patient with head and neck cancer receiving TAK-228 30 mg QW maintained SD for ≥6 months (225 days) (online supplementary figure 3). The overall clinical benefit rate with TAK-228 QW was 45% (9/20 patients). No notable trends were observed between the 20 mg and 30 mg TAK-228 doses.

DISCUSSION

This phase I study was designed to evaluate the safety and tolerability and PK of the investigational oral mTORC1/2 dual inhibitor TAK-228 when administered as milled TAK-228 either as a single agent or in combination with paclitaxel and to assess the effect of dosing conditions (fasted vs fed) on the PK of TAK-228. In addition, the study compared the PK of milled versus unmilled TAK-228 capsules and evaluated the preliminary efficacy

Table 4 Response to TAK-228 given once daily (TAK-228 QD), in combination with paclitaxel (TAK-228+P) or once weekly (TAK-228 QW)

	TAK-228 QD			TAK-228+P			TAK-228 QW		
Patients, n (%)	3 mg (n=11)	4 mg (n=6)	Total (n=17)	4 mg (n=7)	6 mg (n=15)	Total (n=22)	20 mg (n=7)	30 mg (n=13)	Total (n=20)
Best overall response									
CR	0	0	0	0	1 (7)	1 (5)	0	0	0
PR	1 (9)	1 (17)	2 (12)	1 (14)	2 (13)	3 (14)	0	0	0
SD (≥6 months)	3 (27)	1 (17)	4 (24)	0	1 (7)	1 (5)	0	1 (8)	1 (5)
SD (<6 months)	3 (27)	0	3 (18)	2 (29)	4 (27)	6 (27)	3 (43)	5 (39)	8 (40)
PD	3 (27)	3 (50)	6 (35)	2 (29)	5 (33)	7 (32)	3 (43)	3 (23)	6 (30)
No postbaseline scan	1 (9)	1 (17)	2 (12)	2 (29)	2 (13)	4 (18)	1 (14)	4 (31)	5 (25)
Objective response rate (CR+PR)	1 (9)	1 (17)	2 (12)	1 (14)	3 (20)	4 (18)	0	0	0
Clinical benefit rate									
CR+PR + SD	7 (64)	2 (33)	9 (53)	3 (43)	8 (53)	11 (50)	3 (43)	6 (46)	9 (45)
CR+PR + SD ≥6 months	4 (36)	2 (33)	6 (35)	1 (14)	4 (27)	5 (23)	0	1 (8)	1 (5)

CR, complete response; PD, progressive disease; PR, partial response; QD, once daily; QW, once weekly; SD, stable disease; TAK-228+P, TAK-228 once daily 3 days/week+paclitaxel 80 mg/m^2 on days 1, 8 and 15.

of milled TAK-228 capsules. DLTs varied across treatment regimens, with fatigue being the only DLT occurring in more than one patient. There were some similarities in DLTs with TAK-228 between the current and previous studies. In patients with solid tumours, common DLTs were mucosal inflammation, asthaenia, stomatitis, fatigue, rash and hyperglycaemia,[11] whereas patients with haematological malignancies also experienced dose-limiting stomatitis, fatigue, rash, and mucosal inflammation, as well as nausea and vomiting.[7]

MTDs for the milled TAK-228 capsules were determined to be 3 mg (single-agent QD dosing), 6 mg (QD three times per week (QD ×3 days QW) dosing in combination with paclitaxel) and 30 mg (single-agent QW dosing). For single-agent QD and QW TAK-228, the MTDs were also the RP2Ds; however, because patients receiving TAK-228 6 mg in combination arm with paclitaxel often required a reduction in the TAK-228 dose, the RP2D was established as TAK-228 4 mg. At these doses, TAK-228 displayed an acceptable safety profile.

Since it was anticipated that the change to milled TAK-228 capsules may result in faster absorption and a higher maximum plasma concentration than observed with unmilled TAK-228, potentially altering the safety profile, starting doses in this study were generally selected below the current MTD for unmilled TAK-228 in a given treatment regimen. Indeed, the observed MTDs in this study were slightly lower than those previously reported for unmilled TAK-228,[7 11] indicating decreased tolerability for TAK-228 in this study compared with prior studies at the same doses. In a phase I study of 39 patients with multiple myeloma, non-Hodgkin's lymphoma and Waldenström macroglobulinaemia, the MTD for unmilled TAK-228 capsules was determined to be 4 mg QD.[7] In another phase I study of 115 patients with advanced solid tumours, the MTDs were 6 mg QD and 40 mg QW.[11]

The incidence of grade ≥3 drug-related AEs associated with QD milled TAK-228 in this study (59%) is slightly higher than that observed previously using unmilled TAK-228 in haematological malignancies (31%).[7 11] The most commonly reported drug-related AEs, occurring in ≥25% of patients during the treatment period across all treatment arms, were fatigue, diarrhoea and nausea. Patterns of grade ≥3 AEs were also generally similar across the three treatment arms. AEs commonly associated with temsirolimus and everolimus, two mTOR inhibitors approved for clinical use in Europe and the USA, include nausea, rash, stomatitis and fatigue,[13–16] which were also seen in patients taking TAK-228. In contrast, anaemia and thrombocytopaenia, which are commonly associated with temsirolimus and everolimus treatment,[13–17] were not prominent in this study. Hyperglycaemia is a well-documented class effect of mTOR inhibitors, with grade ≥3 events occurring in up to 15% of patients in previous phase III clinical trials[13–15 18] and managed with conventional methods in the clinical setting. We recorded a similar incidence of hyperglycaemia in our study. One death was observed in each arm of the study, but these events were considered unrelated to the study drug by the investigator. In general, the safety profile described here for TAK-228 is consistent with previous studies[7 8 11 19 20] as well as clinical observations of other mTOR inhibitors.[13–18]

PK analyses of TAK-228 revealed that plasma TAK-228 concentration increased in a dose-dependent manner. Consistent with historic data for TAK-228, in the combination arm, paclitaxel infusion 1 day before TAK-228 administration had no meaningful effect on the PK parameters of TAK-228. Administration of milled TAK-228 4 mg

followed by a high-fat meal affected the rate, but not the extent, of TAK-228 absorption. There was an increase in the median T_{max} from 2 hours to 6 hours and a decrease in the geometric mean C_{max} of 40% that was considered clinically meaningful; however, there was no appreciable change in the AUC_{∞}. The decrease in C_{max} when TAK-228 is dosed with a high-fat meal and a change in dosing conditions (ie, requirement for fasted dosing from the previous studies that were flexible and allowed TAK-228 to be taken with food) may collectively help to explain the differences in TAK-228 tolerability observed in this study compared with earlier studies. Interestingly, there were no appreciable differences in the PK of TAK-228 4 mg capsules containing unmilled versus milled formulations when both were administered in a fasted state.

There were no noteworthy trends in efficacy across the different dosing schedules or levels in the study. The solid tumour types responding to single-agent TAK-228 treatment in this study show no striking differences with those reported in previous studies, with the exception of renal cancer.[7 8 11] Indeed, TAK-228 is currently being investigated alone and in combination with the small-molecule PI3K-α inhibitor TAK-117 in patients with advanced or metastatic clear cell renal cell carcinoma (mccRCC).[21]

In the current study, one patient with breast cancer achieved an objective response, while in a previous phase I TAK-228 trial, one patient with breast cancer had SD.[11] These findings are consistent with previous preclinical evaluation of TAK-228 in breast cancer (ML20 and MCF7) xenograft models.[22] Taken together, the efficacy of TAK-228 is in line with positive responses observed for everolimus in breast cancer, temsirolimus in endometrial cancer[23–26] and temsirolimus and everolimus in renal cell carcinoma.[13 14]

Based on the pharmacological properties, safety profile and antitumour efficacy of TAK-228 in the current study, further investigation of TAK-228 as a single agent and in novel treatment combinations is warranted. Currently, a number of phase II clinical studies of TAK-228 are ongoing, including the study previously mentioned, which is investigating TAK-228 QW and QD ×3 days with or without TAK-117 in mccRCC compared with everolimus.[21] Another study is underway to compare TAK-228 QD and QW in combination with fulvestrant versus fulvestrant alone in postmenopausal women with oestrogen receptor-positive/human epidermal growth factor receptor 2-negative (ER-positive/HER2-negative) breast cancer. These other studies, together with the research presented here, will inform dosing considerations and treatment regimens (alone and in combination) for TAK-228.

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