Phase I study of KW-2478, a novel Hsp90 inhibitor, in patients with B-cell malignancies

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Background: KW-2478 is a novel, non-ansamycin, non-purine heat-shock protein 90 (Hsp90) inhibitor.

Methods: In this phase I, multicentre study, KW-2478 was administered intravenously over 1 h at doses ranging from 14 to 176 mg m^{-2} once daily on days 1–5 of a 14-day cycle in a standard 3 + 3 design in 27 patients (22 with multiple myeloma and 5 with non-Hodgkin lymphoma). Patients enrolled had relapsed/refractory disease previously treated with ⩾2 regimens.

Results: There were no dose-limiting toxicities, thus the maximum-tolerated dose was not reached. KW-2478 was well tolerated and did not manifest significant retinal or ocular toxicity. The most common treatment-related adverse events were diarrhoea (33.3%), fatigue (29.6%), headache (25.9%), hypertension (22.2%), nausea (14.8%), vomiting (7.4%), and dizziness (7.4%). Plasma concentrations peaked at the end of infusion and decayed in a biphasic manner with a terminal half-life of ∼6 h. Target inhibition was inferred from the increase in Hsp70 levels in peripheral blood mononuclear cells at doses ⩾71 mg m^{-2}. Twenty-four of 25 (96%) evaluable patients showed stable disease, with five being free of disease progression for ⩾6 months.

Conclusions: Preliminary clinical response data were encouraging and warrant further investigation of KW-2478 in combination regimens for relapsed/refractory B-cell malignancies.

Tangible progress in improving clinical outcome for multiple myeloma (MM) has been primarily driven by the successful introduction of the proteasome inhibitors (e.g. bortezomib) and immunomodulatory drugs (e.g. thalidomide, lenalidomide). However, nearly all patients eventually relapse or become refractory following first- or second-line treatment (Moreau, 2012). These relapsed/refractory MM patients present a therapeutic challenge because of their demonstrated poor clinical outcome (Sinha *et al*, 2012). As a result, novel agents that act in a mechanistically different manner to the proteasome inhibitors and thalidomide derivatives are needed so that they can be used either successively or in combination with standard agents for this difficult-to-treat patient population.

One emerging cellular target in malignancies is heat-shock protein 90 (Hsp90). Heat-shock protein 90, a molecular chaperone required for the stability and function of many cellular proteins, is uniformly expressed in several human MM cell lines (Mitsiades *et al*, 2006; Sharp and Workman, 2006). Heat-shock protein 90 inhibitors induce apoptosis in MM cell lines, disrupt the interaction between bone marrow stromal cells and MM cells, and upregulate other Hsps, including Hsp70 (Banerji *et al*, 2005a; Mitsiades *et al*, 2006; Stühmer *et al*, 2008). A number of Hsp90 inhibitors have shown promise in early clinical trials of haematological and solid tumours but some, such as AUY-922, SNX-5422, and alvespimycin, have been associated with visual disorders including blurred vision, flashes, delayed light/dark

accommodation, and photophobia, possibly due to photoreceptor degeneration and cell death (Zhou et al, 2012). Development of an effective Hsp90 inhibitor with improved overall, and specifically ocular, safety could provide a therapeutic advance for patients with relapsed/refractory MM as well as other malignancies.

KW-2478 is a novel, non-ansamycin, non-purine Hsp90 inhibitor that displays high binding affinity to Hsp90 and potent antitumour activity in in vitro and in vivo models, including activity in primary MM patient samples (Nakashima et al, 2010; Ishii et al, 2012). The activity of KW2478 against primary myeloma cells is retained in the presence of bone marrow stromal cells, suggesting that KW-2478 overcomes the protective effect of the bone marrow microenvironment (Juliger et al, 2008). We designed a phase I study in patients with mature B-cell malignancies including MM, chronic lymphocytic leukaemia (CLL), and non-Hodgkin lymphoma (NHL). The objectives of this study were to determine the maximum-tolerated dose (MTD), safety, and tolerability of KW-2478, characterise its pharmacokinetics and pharmacodynamics, and provide preliminary information on clinical activity.

MATERIALS AND METHODS

Study design. This phase I, open-label, multicentre, dose-escalation study was performed at six institutions in the United Kingdom. The primary objectives were to determine the safety, tolerability, dose-limiting toxicities (DLTs), MTD, and recommended phase II dose (RP2D) of single-agent KW-2478 administered as five consecutive once-daily doses in a 14-day schedule. Secondary objectives were to characterise pharmacokinetics and pharmacodynamics, and report preliminary clinical activity. The protocol, informed consent form, and amendments were reviewed and approved by an Independent Ethics Committee at each study centre. The study was conducted in accordance with the Declaration of Helsinki, International Conference for Harmonisation Good Clinical Practice guidelines, and the Committee for Medicinal Products for Human Use guidelines. All patients provided written informed consent. This study was registered at ClinicalTrials.Gov (NCT00457782).

Eligibility criteria. Patients with confirmed relapsed/refractory MM, CLL, or NHL who had failed $\geqslant 2$ prior standard treatment regimens and had no established therapeutic alternatives were eligible for this study. Patients had to be $\geqslant 18$ years of age, have an Eastern Cooperative Oncology Group (ECOG) performance status of $\leqslant 2$ and a life expectancy $\geqslant 3$ months, and have adequate haematologic, renal, and hepatic function.

Study assessments. Demographics and medical history were recorded at baseline. Safety was monitored through adverse event (AE) monitoring and laboratory assessments. Toxicities were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events v.3.0. Retinal toxicity was monitored by serial visual acuity and colour vision tests before each cycle, together with ophthalmic examination, retinal photograph, electroretinogram (ERG), and autofluorescence imaging at least 21 days before the first dose of study drug, at any significant visual symptom/sign, and at the final study visit. The ERGs incorporated the International Society for Clinical Electrophysiology of Vision standard recordings (Marmor et al, 2004).

Dose-limiting toxicity was defined as any AE considered to be related (possibly, probably, or definitely) to KW-2478, and meeting any of the following criteria during the first cycle of treatment: AE leading to a treatment delay or persisting beyond day 14, grade $\geqslant 4$ non-haematological toxicity or grade 3 non-haematological toxicity deemed clinically significant, grade $\geqslant 4$ haematological toxicity (except those related to cytopenia due to bone marrow

infiltration by primary disease), and $\geqslant 40\%$ change from baseline in ERG or change of ERG deemed clinically significant. The MTD was defined as the highest dose at which at least five of six patients in a cohort did not experience any DLTs. Clinical response was assessed after each or every other cycle, and at the final study visit using standard criteria for MM (Bladé et al, 1998) and NHL (Cheson et al, 1996).

Treatment. KW-2478 (Kyowa Hakko Kirin Co. Ltd, Japan) was administered by intravenous infusion over 1 h at planned doses (14, 28, 47, 71, 99, or 132 mg m^{-2}) on days 1–5 of a 14-day cycle in a standard $3 + 3$ design. As the MTD was not reached at the 132 mg m^{-2} dose, an additional cohort (176 mg m^{-2}) was subsequently added following review of data by the Safety Monitoring Board. Patients could receive KW-2478 either on an outpatient basis or as an in-patient at the investigational site. If outpatient, patients were discharged from the investigational site after the final pharmacokinetic sample (8 h after the fifth dose) and were asked to return to the investigational site on days 9–11. Patients who were not evaluable because of early discontinuation during cycle 1 were replaced. An evaluable patient was defined as a patient who received all five doses of KW-2478 and completed all study visits and procedures by day 14 of cycle 1, unless the patient had a treatment delay or interruption due to toxicity. Patients with a suboptimal response after having completed at least two cycles of treatment and no evidence of progressive disease were given the option to escalate to the highest level for which safety had been established. Each patient was permitted to continue receiving KW-2478 for up to 12 months or until disease progression, unacceptable toxicity, or withdrawal of consent. Haematopoietic growth factors were allowed after the end of cycle 1, as per standard of care. Supportive care, including bisphosphonates and transfusion of packed red blood cells and/or platelets, was also allowed within accepted guidelines. Use of any chemotherapy, radiotherapy, anticancer treatment, or corticosteroid therapy was not permitted during the study.

Pharmacokinetic analyses. Blood samples were collected on days 1 and 5 at the following times: predose, 0.833 h after start of infusion, and 0.167, 0.5, 1, 2, 4, and 8 h after the end of infusion, and predose for days 2 and 4. Heparinised plasma samples were collected, stored at $<-20\,^{\circ}$C, and analysed at an independent central laboratory. Following solid-phase extraction, plasma KW-2478 concentrations were determined using a validated reverse-phase, high-performance liquid chromatography with mass spectrometry/mass spectrometry detection with a lower limit of quantitation of 0.200 mg ml^{-1} The following pharmacokinetic parameters were calculated using a standard non-compartmental model with WinNonlin v.5 (Pharsight Co., Princeton, NJ, USA): maximum observed plasma concentration (C_{max}), areas under the plasma concentration–time curve from time zero to last measurable time point (AUC$_{0-t}$) and to infinity (AUC$_{0-\infty}$), terminal half-life ($t_{1/2}$), elimination rate constant (k_{el}), total plasma clearance (CL), and accumulation ratio (R) comparing day 5 vs day 1 for C_{max} and AUC$_{0-t}$.

Pharmacodynamics. Levels of Hsp70 in peripheral blood mononuclear cells (PBMCs) were examined as a biomarker for Hsp90 inhibition. Peripheral blood mononuclear cells were obtained from blood samples taken before treatment initiation and 8 h postdose on days 1 and 5 of cycle 1. Samples were examined for change in Hsp70 expression using western blot analysis at an independent central laboratory.

Statistical analysis. Adverse events were summarised per dose level and were coded using the Medical Dictionary for Regulatory Activities v.10.0. If more than one AE was recorded for a patient within any system organ class or preferred term, the AE was counted only once in the calculation of percentage of patients with

an event. For patients who dose escalated to a higher dose level, all dose levels of KW-2478 received throughout the duration of an AE were included. Pharmacokinetic and pharmacodynamic data, and clinical responses were summarised descriptively by dose level.

RESULTS

Patient characteristics. A total of 27 patients (22 with MM and 5 with NHL) were recruited to seven different dose cohorts and were treated between 30 April 2007 and 9 September 2010. There were three patients in each of the first five cohorts (14–99 mg m^{-2}), while six patients were recruited in each of the final two cohorts (132 and 176 mg m^{-2}). No patient with NHL was recruited to the first four cohorts. Patient demographic and baseline clinical characteristics are shown in Table 1. Mean age was 63 years (range, 48–75 years), 26 (96.3%) were white, and 17 (63.0%) were male. The majority of patients (88.9%; $n = 24$) had an ECOG performance status of 0 or 1. Of the 22 MM patients, Durie–Salmon classification was stage I ($n = 2$), stage II ($n = 7$), stage III ($n = 9$), and not recorded/not done ($n = 4$). Of the five NHL patients, Ann Arbor classification was stage III ($n = 1$) and stage IV ($n = 4$). All patients had received $\geqslant 2$ prior therapies, with 13 (48.1%) having received $\geqslant 6$ prior therapies. All patients with MM had achieved at least a partial response as best response to prior therapy; one NHL patient had achieved a complete or near-complete response.

All 27 patients received at least one dose of study medication and were included in the safety population. A total of 25 patients (92.6%) were included in the efficacy evaluable population, as two patients (1 each in the 71 and 176 mg m^{-2} cohorts) were excluded as they did not have response assessment.

Study treatment. The mean number of treatment cycles completed was 1.5–3.7 (range, 1–7) at the 14–99 mg m^{-2} doses, 8.0 (range, 2–16) at 132 mg m^{-2}, and 6.7 (range, 1–22) at 176 mg m^{-2}. Eight patients received $\geqslant 6$ cycles, of which five were in the 132 or 176 mg m^{-2} cohorts. No patients required KW-2478 dose reduction, although some patients required dose delays of 10 days or more, generally until AE resolution. Three patients had dose escalation (single step in 2 and two steps in 1).

Recommended phase II dose. There were no DLTs in any dose cohort. As a result, the MTD was not reached. The RP2D was therefore the highest dose tested: 176 mg m^{-2} once daily on days 1–5 every 2 weeks.

Safety. Table 2 summarises AEs reported across all treatment cycles. A total of 282 AEs were reported by 27 patients (100%). The most common AEs were diarrhoea (48.1%), followed by headache (40.7%), rhinitis (40.7%), and fatigue (33.3%). A total of 127 treatment-related AEs were reported by 21 patients (77.8%). The number of treatment-related AEs per patient tended to increase with increasing dose of KW-2478 from an average of 1.0 at the 14 mg m^{-2} dose to 9.2 at the 176 mg m^{-2} dose: duration of exposure to KW-2478 was longer in the higher dose cohorts (see previously), which might partially explain this result. The most common treatment-related AEs per patient were diarrhoea (33.3%), fatigue (29.6%), headache (25.9%), hypertension (22.2%), nausea (14.8%), vomiting (7.4%), and dizziness (7.4%). Although hypertension was a common treatment-related AE, there was no apparent dose-related increase in mean blood pressure for all patients during the study.

Most AEs were grade 1 or 2. Ten patients (37.0%) experienced a total of 19 grade 3/4 AEs, of which five events in three patients were considered possibly treatment-related (two episodes of lethargy and one of syncope; QT prolongation; and neutrophil decrease in respective patients). Two patients died during the study (one after study completion), but both deaths were considered unrelated to study medication (bronchopneumonia and lower respiratory tract infection, respectively).

Overall, six patients (22.2%) experienced nine AEs classified as eye disorders, which were considered possibly related to KW-2478 in four patients: decreased visual acuity (grade 1 in one patient on 28 mg m^{-2}), blurred vision (grades 1 and 2 in two patients on 71 mg m^{-2}), and dry eyes (grade 2 in one patient on 176 mg m^{-2}) – all resolved except for dry eyes, which also required treatment. There were no clinically significant abnormalities on routine ophthalmological examination nor fundus autofluorescence imaging. There were no notable effects on visual acuity nor colour vision. In summary, there were no clinically meaningful changes in ophthalmic parameters.

There were no clinically meaningful changes in haematological or biochemistry values, vital signs, body weight, nor ECG results.

Pharmacokinetics. The pharmacokinetics of KW-2478 are summarised in Table 3. Mean C_{max} and AUC values increased linearly in relation to dose on days 1 and 5, and accumulation ratios for these parameters indicated no relevant accumulation from days 1 to 5.

Table 1. Baseline demographic and clinical characteristics								
	KW-2478 dose level (mg m^{-2})							
Characteristic	14 ($n = 3$)	28 ($n = 3$)	47 ($n = 3$)	71 ($n = 3$)	99 ($n = 3$)	132 ($n = 6$)	176 ($n = 6$)	Total ($N = 27$)
Mean age (years) (range)	64.3 (58–68)	70.0 (66–74)	64.3 (63–65)	66.7 (55–75)	57.0 (48–62)	60.7 (55–65)	59.7 (52–69)	62.6 (48–75)
Mean BSA (m^2) (range)	1.7 (1.7–1.8)	1.8 (1.6–2.1)	1.9 (1.8–2.0)	1.9 (1.8–2.2)	2.0 (1.9–2.0)	2.0 (1.8–2.1)	1.9 (1.6–2.1)	1.9 (1.6–2.2)
Gender, n (%)								
Male	1 (33.3)	1 (33.3)	2 (66.7)	2 (66.7)	2 (66.7)	5 (83.3)	4 (66.7)	17 (63.0)
Female	2 (66.7)	2 (66.7)	1 (33.3)	1 (33.3)	1 (33.3)	1 (16.7)	2 (33.3)	10 (37.0)
Race, n (%)								
Caucasian	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	5 (83.3)	6 (100.0)	26 (96.3)
Asian	0	0	0	0	0	1 (16.7)	0	1 (3.7)
Disease, n (%)								
MM	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	2 (66.7)	4 (66.7)	4 (66.7)	22 (81.5)
NHL	0	0	0	0	1 (33.3)	2 (33.3)	2 (33.3)	5 (18.5)
ECOG performance status, n (%)								
0	2 (66.7)	1 (33.3)	2 (66.7)	2 (66.7)	3 (100.0)	4 (66.7)	4 (66.7)	18 (66.7)
1	1 (33.3)	1 (33.3)	1 (33.3)	0	0	2 (33.3)	1 (16.7)	6 (22.2)
2	0	1 (33.3)	0	1 (33.3)	0	0	1 (16.7)	3 (11.1)
Abbreviations: BSA = body surface area; ECOG = Eastern Cooperative Oncology Group; MM = multiple myeloma; NHL = non-Hodgkin lymphoma.								

Table 2. Treatment-emergent adverse events

	No. of patients (%)						
	KW-2478 dose level (mg m^{-2})[a]						
	14 ($n=3$)	**28 ($n=3$)**	**47 ($n=4$)**	**71 ($n=4$)**	**99 ($n=4$)**	**132 ($n=6$)**	**176 ($n=7$)**
AE	3 (100.0)	3 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	6 (100.0)	7 (100.0)
AE grade 3/4	0	1 (33.0)	1 (25.0)	2 (50.0)	1 (25.0)	3 (50.0)	2 (28.6)
Treatment-related AE	2 (66.7)	2 (66.7)	3 (75.0)	4 (100.0)	3 (75.0)	5 (83.3)	5 (71.4)
Serious AE	0	0	0	2 (50)	2 (50)	3 (50.0)	1 (14.3)
Death[b]	0	0	0	1 (25)	0	0	0
Any AE occurring in >2 patients overall by preferred term							
Diarrhoea	0	0	2 (50.0)	2 (50.0)	2 (50.0)	3 (50.0)	4 (57.1)
Rhinitis	1 (33.3)	1 (33.3)	1 (25.0)	1 (25.0)	0	4 (66.7)	3 (42.9)
Headache	1 (33.3)	0	0	2 (50.0)	2 (50.0)	4 (66.7)	2 (28.6)
Fatigue	1 (33.3)	0	0	1 (25.0)	2 (50.0)	1 (16.7)	4 (57.1)
Nausea	1 (33.3)	1 (33.3)	0	2 (50.0)	1 (25.0)	2 (33.3)	0
Vomiting	0	1 (33.3)	1 (25.0)		1 (25.0)	3 (50.0)	1 (14.3)
Hypertension	0	0	2 (50.0)	2 (50.0)	1 (25.0)	2 (33.3)	0
URTI	0	0	2 (50.0)	2 (50.0)	0	0	2 (28.6)
Back pain	1 (33.3)	1 (33.3)	0	0	2 (50.0)	1 (16.7)	0
Anaemia	0	0	1 (25.0)	0	0	0	3 (42.9)
Oral herpes	0	1 (33.3)	0	1 (25.0)	1 (25.0)	0	1 (14.3)
LRTI	0	0	0	1 (25.0)	0	1 (16.7)	2 (28.6)
Musculoskeletal chest pain	0	0	0	0	1 (25.0)	2 (33.3)	1 (14.3)
Dizziness	0	0	0	2 (50.0)	0	1 (16.7)	0
Abdominal pain	0	0	0	0	1 (25.0)	2 (33.3)	0
Anorexia	0	0	0	0	0	1 (16.7)	2 (28.6)
Acne	0	1 (33.3)	1 (25.0)	1 (25.0)	0	0	0
Hair disorder	0	1 (33.3)	1 (25.0)	1 (25.0)	0	0	0
Herpes simplex infection	0	1 (33.3)	0	1 (25.0)	0	0	1 (14.3)
Haemoglobin decreased	0	0	0	1 (25.0)	0	2 (33.3)	0

Abbreviations: AE = adverse event; LRTI = lower respiratory tract infection; URTI = upper respiratory tract infection.
[a]Patients with dose escalations are counted in all relevant dose groups.
[b]Both were considered unrelated to medication (bronchopneumonia at 71 mg m^{-2} and LRTI at 99 mg m^{-2}).

Table 3. Pharmacokinetic parameters for KW-2478

		Mean ± s.d.						
		KW-2478 dose level (mg m^{-2})						
Parameter	**Day**	**14 ($n=3$)**	**28 ($n=3$)**	**47 ($n=3$)[a]**	**71 ($n=3$)[a]**	**99 ($n=3$)**	**132 ($n=6$)**	**176 ($n=6$)[b]**
C_{max} (ng ml^{-1})	1	645 ± 306	1620 ± 556	1990 ± 236	2620 ± 585	3400 ± 876	5410 ± 1622	6838 ± 1961
	5	656 ± 342	1320 ± 531	1705 ± 785	2675 ± 49.5	2977 ± 610	5972 ± 940	6062 ± 1411
R_{Cmax}	5	1.02 ± 0.19	0.81 ± 0.12	0.86 ± 0.27	0.93 ± 0.15	0.88 ± 0.06	1.17 ± 0.34	0.97 ± 0.07
AUC_{0-t} (ng h^{-1} ml^{-1})	1	819 ± 403	1935 ± 848	2325 ± 652	2798 ± 432	4220 ± 1251	6910 ± 1864	10 197 ± 4307
	5	844 ± 445	1639 ± 768	2334 ± 532	3681 ± 497	3673 ± 932	7262 ± 1331	8355 ± 2496
R_{AUC}	5	1.02 ± 0.06	0.86 ± 0.12	1.00 ± 0.15	1.22 ± 0.23	0.88 ± 0.03	1.08 ± 0.17	0.95 ± 0.10
$AUC_{0-\infty}$ (ng h^{-1} ml^{-1})	1	992 ± 497	2353 ± 1117	2758 ± 891	3197 ± 400	4826 ± 1504	7876 ± 2216	11 501 ± 5006
$t_{1/2}$ (h)	1	6.05 ± 0.37	6.48 ± 0.96	6.60 ± 0.69	6.29 ± 0.93	5.83 ± 0.14	5.71 ± 0.61	5.43 ± 0.39
k_{el} (l h^{-1})	1	0.11 ± 0.01	0.11 ± 0.02	0.11 ± 0.01	0.11 ± 0.02	0.12 ± 0.003	0.12 ± 0.01	0.13 ± 0.01
CL (l h^{-1})	1	28.9 ± 11.1	23.1 ± 15.9	34.9 ± 8.35	43.0 ± 6.80	42.7 ± 12.5	35.0 ± 9.99	32.8 ± 10.7

Abbreviations: AUC_{0-t} = area under plasma concentration-time curve from time zero to the last measurable time point; $AUC_{0-\infty}$ = AUC from time zero to infinity; CL = total plasma clearance; C_{max} = maximum observed plasma concentration; k_e = elimination rate constant; R_{Cmax} = accumulation ratio of C_{max}; R_{AUC} = accumulation ratio of AUC; $t_{1/2}$ = terminal half-life.
[a]$n=2$ on day 5.
[b]$n=5$ on day 5.

Mean $t_{1/2}$ (range, 5.4–6.6 h) and total CL (range, 23.1–43.0 l h^{-1}) appeared independent of dose. Plasma concentrations over time profiles for KW-2478 on days 1 and 5 are shown in Figure 1. Plasma concentration declined in a biphasic manner with a rapid distribution phase and slower elimination phase.

Pharmacodynamics. Induction of Hsp70 was measured in PBMCs of patients treated with KW-2478 as a surrogate marker of Hsp inhibition. A representative western blot in a patient who received KW-2478 176 mg m^{-2} shows Hsp70 induction 8 h after the first dose on day 1, a return to normal on day 5 predose, and renewed induction 8 h after dosing on day 5 (Figure 2A). Heat-shock protein 70 induction of ≥25% above baseline was consistently noted at doses ≥71 mg m^{-2} at 8 h after dosing on days 1 and 5, with some evidence of upregulation at lower doses, particularly on day 5 (Figure 2B).

Tumour response. Best tumour response over all cycles was assessed among 21 MM patients and four NHL patients. No patients achieved complete or partial responses. Among the 21
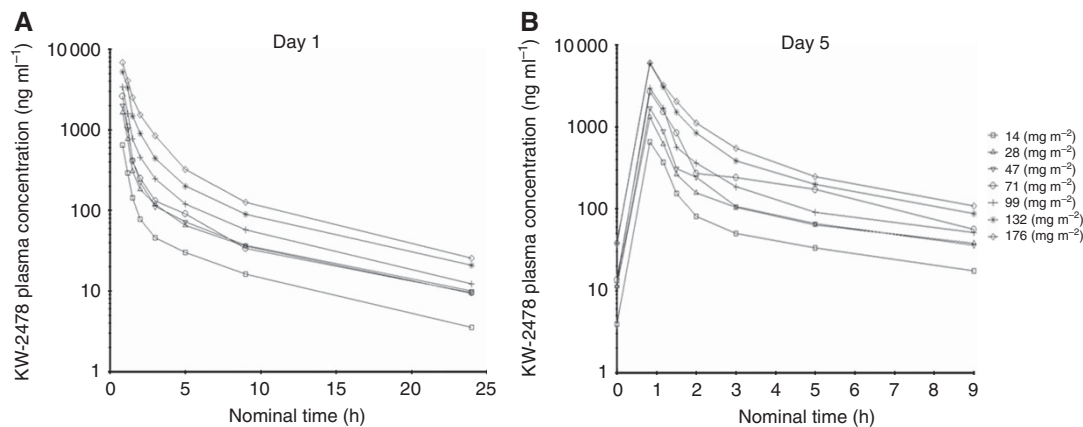
Figure 1. Mean plasma KW-2478 concentration over time on days 1 and 5.
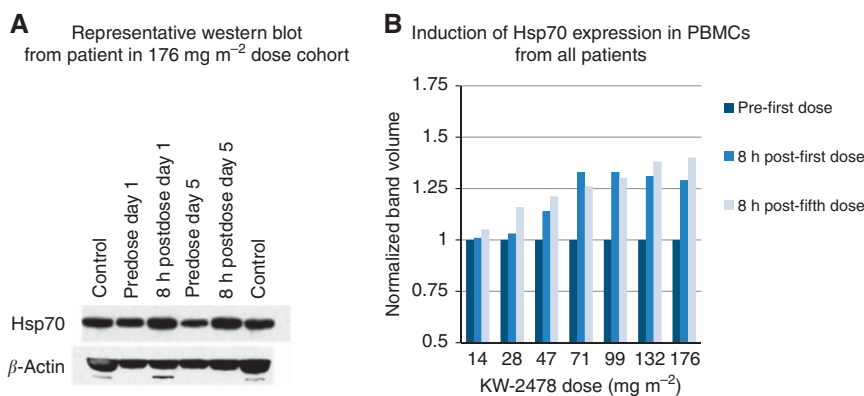

Figure 2. Hsp70 expression. (A) Representative western blot for Hsp70 expression from a patient receiving KW-2478 176 mg m^{-2}. (B) Changes in mean Hsp70 expression in peripheral blood monocytes at different KW-2478 dose levels.

evaluable MM patients, 20 (95.2%) had stable disease (SD) and one (4.8%) had progressive disease. All four evaluable NHL patients (100%) had SD. This gives an overall disease control rate of 96.0%. Eight patients had SD lasting $\geqslant 3$ months and five had SD lasting $\geqslant 6$ months, including one patient in each of the 132 and 176 mg m^{-2} cohorts with SD sustained for 12.9 and 11.9 months, respectively. All but one of these patients with a more durable ($\geqslant 6$ months) SD received doses $\geqslant 71$ mg m^{-2}. Details of individual patient responses, duration of treatment, and reasons for discontinuation are given in Table 4.

DISCUSSION

Patients with refractory MM are inherently difficult to treat and have considerable unmet need for novel treatment options. In this phase I study of the novel Hsp90 inhibitor, KW2478, as single-agent therapy, a disease stabilisation rate of 96% in a heavily pretreated study population with resistant disease and no other therapeutic options is notable. In such patients with progressive MM, sustained SD could be considered clinically meaningful. Eight patients had SD lasting for $\geqslant 3$ months and five patients had SD lasting for $\geqslant 6$ months: this included two patients, one in the 132 mg m^{-2} and one in the 176 mg m^{-2} groups who had SD lasting for 12.9 and 11.9 months, respectively. Furthermore, all but one of these patients with a more durable SD occurred at doses $\geqslant 71$ mg m^{-2}, that is, at doses showing demonstrable Hsp70 induction. Frequent and sustained SD is an encouraging finding for patients who had already failed $\geqslant 2$ prior regimens. The use of Hsp70 expression as a biomarker for inhibition of Hsp90 has been validated by Banerji et al (2005b), who showed that induction of

Hsp70 levels occurs with Hsp90 inhibitors at concentrations required for clinical efficacy. Heat-shock protein 70 induction was consistently demonstrated at KW-2478 doses $\geqslant 71$ mg m^{-2} in our current study.

Proteasome inhibitors (bortezomib carfilzomib) represent a significant advance in the treatment of MM, yet not all patients respond and disease responses are often short lived, especially in the relapsed/refractory setting. Overcoming disease resistance to proteasome inhibition is therefore the focus of intense study. Among the cellular responses to proteasome inhibition is Hsp upregulation, indicating a state of cellular stress (Mitsiades et al, 2002). Thus, Hsp90 inhibitors may act to sensitise cells to the activity of agents such as bortezomib (Mitsiades et al, 2011). There are no apparent significant overlapping toxicities between KW-2478 and bortezomib, as well as no apparent neurotoxicity nor significant thrombocytopenia with KW-2478. Unlike some other members of the Hsp90 inhibitor class (Rajan et al, 2011; Sessa et al, 2013), there was no clinically significant ocular toxicity. Thus, there is a sound basis for the rational combination of KW-2478 with proteasome inhibition.

In conclusion, the RP2D for single-agent KW-2478 is 176 mg m^{-2} on days 1–5 every 14 days in patients with relapsed/refractory B-cell malignancies. KW-2478 was safe and well tolerated, with no DLTs or unexpected toxicities being detected. Treatment-related AEs $\geqslant$ grade 3 were rare. Indication of clinical activity was suggested by sustained SD in some patients. Further clinical study of KW-2478 is therefore warranted. The combination of KW-2478 and bortezomib is therefore being investigated in a phase I/II study in patients with refractory/relapsed MM (NCT01063907).

Table 4. Duration of response in the efficacy evaluable population (N = 25)

Dose level	Patient no.	Diagnosis	Best response	Cycle (end of) at which best response observed	Duration of response (months)	No. of cycles received (cycle = 2 weeks)	Reason for end of treatment
14 mg m^{-2}	102	MM	SD	1	1	1	Withdrawal of consent
	104	MM	SD	1	1	2	Disease progression
	105	MM	SD	1	1.4	5	Disease progression
28 mg m^{-2}	106	MM	SD	1	11.3	22	Disease progression
	401	MM	SD	1	0.8	3	Disease progression
	501	MM	SD	1	1.8	2	Disease progression
47 mg m^{-2}	404	MM	SD	1	1	2	Disease progression
	405	MM	SD	1	1.3	3	Disease progression
	406	MM	SD	1	1.3	5	Disease progression
71 mg m^{-2}	201	MM	Not done[a]	N/A	N/A	3	Adverse event
	407	MM	SD	1	1.1	3	Disease progression
	408	MM	SD	1	5.1	11	Disease progression
99 mg m^{-2}	301	NHL	SD	1	1.5	4	Disease progression
	410	MM	SD	1	3.1	8	Disease progression
132 mg m^{-2}	202	MM	SD[b]	2	1.4	4	Disease progression
	203	MM	SD	1	8.8	18	Disease progression
	411	NHL	SD	1	1.4	4	Disease progression
	412	NHL	SD[b]	2	8.2	17	Disease progression
	413	MM	SD	1	1.4	3	Disease progression
	414	MM	SD	1	12.9	26	12 months treatment completed
176 mg m^{-2}	109	MM	SD	1	3.5	7	Withdrawal of consent
	302	NHL	SD	1	1.3	3	Withdrawal of consent
	416	MM	SD	1	1.3	3	Adverse event
	417	MM	SD	1	11.9	24	Disease progression
	602	MM	SD	1	2	5	Disease progression

Abbreviations: MM = multiple myeloma; N/A = not applicable; NHL = non-Hodgkin lymphoma; SD = stable disease.
[a]Efficacy evaluation was not carried out for any cycle.
[b]No efficacy evaluation was carried out at the end of cycle 1.

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CONFLICT OF INTEREST

D Nakashima and S Akinaga are employees of Kyowa Hakko Kirin Co. Ltd (Tokyo and USA). The other authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed equally to data analysis and interpretation, figure creation, and writing of the manuscript.

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