

Cancer Therapy: Clinical

The First-in-Human Study of the Hydrogen Sulfate (Hyd-Sulfate) Capsule of the MEK1/2 Inhibitor AZD6244 (ARRY-142886): A Phase I Open-Label Multicenter Trial in Patients with Advanced Cancer

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Abstract

Purpose: In part A, the aim was to define the maximum tolerated dose (MTD) of the hydrogen sulfate (Hyd-Sulfate) oral capsule formulation of the mitogen-activated protein kinase kinase inhibitor AZD6244 (ARRY-142886). In part B, the aim was to compare the pharmacokinetic profile of the new Hyd-Sulfate capsule with the initial AZD6244 free-base suspension and further characterize the pharmacodynamic profile and efficacy of the new formulation.

Experimental Design: In part A, 30 patients received escalating doses of AZD6244 Hyd-Sulfate twice daily. In part B, 29 patients were randomized to a single dose of the Hyd-Sulfate capsule or free-base suspension, followed by a washout, then a single dose of the alternative formulation. Patients received the Hyd-Sulfate capsule twice daily at MTD of part A thereafter.

Results: The MTD of the Hyd-Sulfate capsule was 75 mg twice daily. Dose limiting toxicities were Common Terminology Criteria for Adverse Events grade 3 acneiform rash and pleural effusion. Fatigue (65.7%) and acneiform dermatitis (60.0%) were the most frequent adverse events at the MTD. Based on area under curve₀₋₂₄, exposure of the 75 mg Hyd-Sulfate capsule relative to the 100 mg free-base suspension was 197% (90% confidence interval, 161-242%). Pharmacodynamic analysis showed that inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced extracellular signal-regulated kinase phosphorylation in peripheral blood lymphocytes was related to plasma concentrations of AZD6244, with an estimated IC₅₀ of 352 ng/mL and maximum inhibition (E_{max}) of ~91%, showing target inhibition. A patient with metastatic melanoma bearing a V600E *BRAF* mutation achieved a complete response persisting after 15 months of therapy.

Conclusions: The AZD6244 Hyd-Sulfate capsule formulation has shown a favorable toxicity, pharmacokinetic, and pharmacodynamic profile, and is being taken forward in ongoing clinical trials. *Clin Cancer Res*; 16(5); 1613-23. ©2010 AACR.

The Ras/Raf/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway is a key signaling cascade involved in the regulation of cell cycle, proliferation, differentiation, and survival.

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Dysregulation of this pathway as a result of aberrant upstream growth factor receptor signaling (1), or by activating mutations in signaling molecules such as Ras and Raf themselves, has been implicated in tumor formation (2).

MEK1/2 are central components of the Ras/Raf/MEK/ERK pathway. They lie downstream of Ras and Raf and are attractive anticancer targets because inhibition of MEK1/2 can potentially block inappropriate signal transduction, originating from the cell surface or due to Ras/Raf mutations (1-4).

AZD6244 is a potent, selective, allosteric inhibitor of MEK1/2 that has shown activity in a number of cell-based growth assays and a variety of human tumor mouse xenograft models, including non-small cell lung cancer (NSCLC) and melanoma (5-7). The clinical efficacy of an oral free-base suspension of AZD6244 has been shown in a phase I trial in patients with advanced solid malignancies, which showed it to be well tolerated, with target

Translational Relevance

Mitogen-activated protein kinase kinase (MEK) is a central component of the Ras/Raf/MEK/extracellular signal-regulated kinase pathway, which is known to be deregulated in a significant number of cancers. AZD6244 is an oral MEK inhibitor in an advanced stage of clinical development. Previous phase I trials of AZD6244 (ARRY-142886) used a liquid suspension that established a proof of concept but would not be practical for long-term twice-daily dosing. This study defines the safety, pharmacokinetic, and pharmacodynamic profile of an AZD6244 hydrogen sulfate capsule that is being taken forward into ongoing phase I/phase II monotherapy studies and combination phase II studies.

inhibition observed at the recommended phase II dose of 100 mg twice daily (8). The clinical activity of the free-base suspension has also been shown in phase II monotherapy studies (9, 10).

To enable more convenient dosing of AZD6244, a solid oral capsule formulation incorporating a hydrogen sulfate (Hyd-Sulfate) salt has been developed. Here, we report a first-in-human, two-part phase I study evaluating the safety, tolerability, efficacy, pharmacokinetics, and pharmacodynamics of the Hyd-Sulfate capsule in patients with advanced cancers. Part A of the study established the maximum tolerated dose (MTD) of the Hyd-Sulfate capsule and part B compared the exposure and relative oral bioavailability of this formulation with the oral free-base suspension.

Patients and Methods

Aims

The primary objectives of the study were to assess the safety and tolerability of the AZD6244 Hyd-Sulfate capsule formulation (the chemical structure of AZD6244 is provided in Supplementary Fig. S1). Secondary objectives were to investigate the pharmacokinetics of the Hyd-Sulfate capsule following single and multiple oral dosing and (in part B only) to determine the relative oral bioavailability of the Hyd-Sulfate capsule to the free-base suspension. Exploratory objectives were assessment of pharmacodynamic activity and clinical efficacy.

Patient selection

For parts A and B, patients (age >18 y, WHO performance status 0-2), refractory to standard therapies or for whom no conventional therapies exist, were enrolled from two centers in the Netherlands, one in the United Kingdom, and one in the United States. Other inclusion criteria, based on blood chemistry, were comparable with those described in the phase I trial of the free-base suspension (8).

Experimental treatment

This study was done in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with International Conference on Harmonization/Good Clinical Practice. All patients provided informed consent.

Part A. At least six evaluable patients were recruited into each cohort to determine the MTD (defined as the last dose assessed below the nontolerated dose). Each patient received a single dose of AZD6244 Hyd-Sulfate capsule on day 1, followed by continuous twice-daily dosing from day 2 onwards. The start dose in the first cohort was 25 mg and was predicted to produce a maximum plasma concentration (C_{max}) equal to or less than previously observed following dosing with 100 mg free-base suspension (8).

Dose-limiting toxicity (DLT) was defined as occurrence of any of the following toxicities in the first 22 d of dosing: (a) any Common Terminology Criteria for Adverse Events (CTCAE) grade 4 hematologic toxicity, CTCAE grade 3 neutropenia with fever, or thrombocytopenia associated with bleeding; (b) any nonhematologic CTCAE grade 3/4 toxicity despite adequate supportive care that was not clearly related to underlying disease; or (c) any persistent nonhematologic CTCAE grade 2 toxicity despite optimal therapy or any dose interruption >2 wk for an adverse event (AE) considered related to AZD6244 treatment.

Doses that produced a DLT in ≤ 1 of 6 patients were defined as tolerated and dose escalation continued. Doses at which DLTs were observed in ≥ 2 of 6 patients were defined as nontolerable and dose escalation was stopped. If a patient discontinued or did not complete $\geq 80\%$ of the defined doses of AZD6244 for non-DLT reasons prior to completion of the first 22-d cycle, they were replaced by another patient to ensure ≥ 6 evaluable patients were treated at that dose level.

Part B. Part B commenced when the MTD in part A had been defined (Fig. 1). In part B, patients were randomized on day 1 to a single 75 mg dose of the Hyd-Sulfate capsule or 100 mg free-base suspension. These doses were the MTD for each formulation. After a 7-d washout period, patients received a single dose of the alternative formulation on day 8. Following the relative bioavailability assessment, patients received the Hyd-Sulfate capsule twice daily continuously from day 9 onwards, until discontinuation criteria were met.

Parts A and B. Patients could continue to receive AZD6244 until disease progression and provided they continued to derive benefit from treatment. Patients who continued to receive treatment beyond the defined end of study (the date when all patients still receiving treatment had been followed up for a minimum period of 6 mo) were followed up according to the investigational site standard of care and investigator judgment. Investigators were required to report all serious AEs (SAE) until 30 d after study treatment was discontinued.

Plasma pharmacokinetic profiles for parts A and B were obtained on days 1 and 8. C_{max} and time to maximum plasma concentration (t_{max}) were derived from the plasma

concentration-time profile. The area under the concentration-time curve (AUC), terminal half-life ($t_{1/2}$), clearance (CL/F), and volume of distribution (V_{ss}/F) were calculated using noncompartmental methods. Full details of these methods are provided as supplementary material. In part B, the relative oral bioavailability of the Hyd-Sulfate capsule to the free-base suspension was assessed in addition to comparing the exposures of the formulations at the MTD doses. Plasma concentrations of the active circulating metabolite of AZD6244 (*N*-desmethyl AZD6244, which is 3- to 5-fold more potent *in vitro* than AZD6244) were also determined in parts A and B.

Inhibition of ERK phosphorylation in *ex-vivo* 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-treated peripheral blood cells was used as a surrogate pharmacodynamic biomarker for inhibition of MEK1/2 in all patients on day 1 in part A and on days 1 and 8 in part B (pretreatment and 1, 4, 8 and 24 h postdose). Full details of this method are provided as supplementary material.

Assessments

Tumor assessments were done every 8 wk \pm 7 d relative to day 1. Disease progression was assessed using Response Evaluation Criteria in Solid Tumors (RECIST 1.0) if measurable, or other assessment if nonmeasurable. AEs were evaluated throughout the study (at weeks 1, 2, and 3, and then every 4 wk until withdrawal from treatment) and until 30 d after discontinuation of study drug. All AEs were graded according to CTCAE, version 3.0. Complete ophthalmologic exams were done within 14 d of day 1 and again between weeks 4 and 6, and on occurrence of visual disturbance AEs. Electrocardiogram (ECG) measurements were captured in triplicate predose, 2 h postdose on day 1, and 2 h postdose on day 8. ECGs were also done in triplicate at withdrawal from treatment and in the event of any cardiorespiratory AEs.

Statistical evaluation

To determine the relative oral bioavailability of the Hyd-Sulfate capsule to the free-base suspension and to compare the exposures of the formulations at the MTD doses, statistical analyses from nominal days 1 and 8 were carried out for AUC₀₋₂₄ and C_{max} in part B. Data were evaluated by ANOVA, allowing for effect of formulation, period in which formulations were received (i.e., day 1 or day 8), sequence of formulations received, and subject within sequence (as a random effect). Prior to estimating relative oral bioavailability only, data were dose-normalized. The ratio of the geometric least square means (glsmeans) for the Hyd-Sulfate capsule to the free-base suspension and 90% confidence interval (90% CI) were estimated.

Results

Patient demographics and baseline disease characteristics for parts A and B of the study are shown in Table 1.

Safety and tolerability (Parts A and B)

The most frequently reported AEs, regardless of dose, severity, causality, or seriousness, for the 75-mg cohort of parts A and B were fatigue, acneiform dermatitis, nausea, diarrhea, and peripheral edema (Table 2). Time to onset of the most frequently occurring AEs was approximately 29 days for fatigue, 18 days for acneiform dermatitis, 22 days for nausea, 12 days for diarrhea, and 43 days for peripheral edema. Less frequently occurring adverse events are reported in Supplementary Table S1.

Dermatologic AEs. A number of different dermatologic conditions were reported in this study, and their onset followed a distinct temporal pattern. The most frequently occurring dermatologic AEs that started during the first month on treatment were dermatitis acneiform, rash (maculo-papular, erythematous, macular, papular, exfoliative), erythema, skin exfoliation, folliculitis, and erysipelas, in decreasing order of frequency. Dermatologic AEs reported as starting after more prolonged administration (>3 months on treatment) were dry skin, pruritis, skin fissures, and paronychia.

Gastrointestinal AEs. AEs of diarrhea, nausea, and vomiting were commonly reported in this study, most of which were CTCAE grade 1, and were causally related to study treatment. Most diarrhea events started within the first 2 weeks of twice-daily dosing (in some instances after only one dose of AZD6244). However, the temporal relationship between onset of nausea and vomiting and the start of AZD6244 dosing was weaker, generally starting slightly later after the onset of dosing with AZD6244 than diarrhea AEs. Approximately one third of these patients required concomitant loperamide to manage diarrhea events. Four of 56 patients (7.1%) required a serotonin 5HT₃ receptor antagonist to control nausea.

Fluid accumulation events. Edema was reported under a variety of terms in this study, of which peripheral edema was the most frequent. Most AEs of peripheral edema were CTCAE grade 1 and the majority were considered to be related to study treatment. Onset of peripheral edema events was commonly several weeks into treatment with AZD6244. Some patients required initiation of diuretic therapy to control the peripheral edema. Other less frequently reported edema terms in this study included face edema, periorbital edema, pitting edema, eyelid edema, and localized edema. In addition, eye swelling, which may also represent an edema-type event, was also reported. Other AEs relating to possible fluid accumulation syndromes (pleural effusion and ascites) were also reported. No events of edema led to discontinuation of study treatment.

Visual function AEs. Ten patients in the study had visual function AEs [75 mg ($n = 9$) and 100 mg ($n = 1$)], the most common of which was blurred vision ($n = 4$). Less frequent visual function AEs included diplopia, dry eye, eyelid edema, increased lacrimation, visual disturbance, and scleral hemorrhage. Most visual AEs were CTCAE grade 1 in intensity. One CTCAE grade 3 AE of blurred vision was reported in the 75-mg-dose cohort that started

Table 1. Demographic and baseline disease characteristics of patients: full analysis set

	Number of patients (%)	
	Part A, n = 30	Part B, n = 29
Sex		
Male	20 (66.7)	13 (44.8)
Female	10 (33.3)	16 (55.2)
Age (y)		
Mean (SD)	57.9 (10.9)	57.1 (13.5)
Range	30-76	26-9
Racial origin		
Caucasian	30 (100.0)	27 (93.1)
Black	0 (0)	1 (3.4)
Mixed race	0 (0)	1 (3.4)
WHO performance status*		
0	14 (46.7)	11 (37.9)
1	14 (46.7)	16 (55.2)
2	1 (3.3)	1 (3.4)
Unknown	1 (3.3)	1 (3.4)
Primary tumor location [†]		
Colorectal	9 (30.0)	11 (37.9)
Lung	3 (10.0)	2 (6.9)
Esophagus	2 (6.7)	0 (0)
Skin/soft tissue	10 (33.3)	4 (13.8)
Stomach	0 (0)	2 (6.9)
Pancreas	0 (0)	2 (6.9)
Other	0 (0)	2 (6.9)
Previous treatments		
Radiotherapy	10 (33.3)	9 (31.0)
Chemotherapy	28 (93.3)	26 (89.7)
Immunotherapy	12 (40.0)	7 (24.1)
Other	13 (43.3)	8 (27.6)

*WHO performance status: 0, normal activity; 1, restricted activity; 2, in bed \leq 50% to 100% bedridden.

[†]Only those primary tumor locations reported for two or more patients are shown.

on day 2 of treatment, and resolved fully without intervention 4 days later. One grade 2 event of visual disturbance led to withdrawal from the 100-mg dose. However, this AE occurred on the same day as AEs of nausea, fungal urinary tract infection, anorexia, and acute renal failure, all of which were classified as leading to withdrawal of study treatment. No pattern of underlying visual abnormalities was identified following ophthalmologic examination.

Corrected Qt interval (QTc) prolongation events. A review of the ECG parameters showed no consistent trend of significant QTcF method prolongation across the dose cohorts in part A; the maximum increase in mean QTc-Fredericia method was noted in the 75-mg twice-daily cohort with a 12.9 ms (SD, 13.2; range -1 to +27) increase 2 hours postdose on day 8. No consistent trend in QTcF was identified when comparing the results obtained in part B after administration of single doses of both formulations. There were no individual QTcF values on treatment >500 ms, and no individual increases of QTcF of >60 ms. Due to

the limited cohort size no definitive statement on liability for QTcF prolongation is possible based on the data collected in this study.

SAEs and AEs of CTCAE grade 3 or above. Twelve of 28 patients (42.9%) in part A and 9 of 28 patients (32.1%) in part B reported a SAE. The most commonly reported SAE was vomiting ($n = 4$; only one of which was considered treatment related). Other SAEs reported by >1 patient were lower respiratory tract infection ($n = 3$), pyrexia ($n = 2$), and hypertension ($n = 2$; both attributed to treatment). Three SAEs reported in part A had an outcome of death, none of which were considered treatment related by the investigators. These included sepsis (25-mg cohort, $n = 1$), pulmonary embolism (75-mg cohort, $n = 1$), and respiratory failure (75-mg cohort, $n = 1$).

Thirteen of 28 (46.4%) patients in part A and 20 of 28 (71.4%) patients in part B reported an AE of CTCAE grade 3 or higher, the majority of which were reported by only one patient. Events that were reported by more than one patient were left ventricular dysfunction, nausea,

vomiting, fatigue, febrile infection, lower respiratory tract infection, increase in blood alkaline phosphate, increase in γ -glutamyltransferase, hypoxia, acneiform dermatitis, and hypertension. Of these, fatigue was the most commonly reported CTCAE grade 3 or higher AE, reported in eight patients.

Two DLTs were reported in the 100-mg cohort in part A within the first 22 days of dosing (pleural effusion and acneiform dermatitis; Table 3) that led to treatment discontinuation. The patient with pleural effusion was a 76-year-old female with locally advanced lung cancer, who had CTCAE grade 1 pleural effusion at study onset. CTCAE grade 3 pleural effusion was reported as an SAE 18 days after onset of study treatment. The patient was subsequently hospitalized for three days, and the dose reduced to 75 mg twice daily. Pleural effusion was not considered disease progression by the investigator and the patient went on to receive treatment at the reduced dose until she had radiologic evidence of disease progression at day 80.

Dose reductions and interruptions. Two patients receiving 100 mg twice daily Hyd-Sulfate capsule in part A required a dose reduction due to AEs but both remained on 75 mg

twice daily until disease progression. Five of 28 patients (17.9%) in part A and 5 of 28 patients (17.9%) in part B had an AE that led to a dose interruption.

Laboratory and clinical parameters. Increases in serum liver transaminases were observed within 1 week of initiating treatment (usually up to 1 CTCAE grade) which stabilized within the first 28 days, except in patients at time points immediately prior to withdrawal due to disease progression. Assessment of left ventricular ejection fraction at baseline after 8 weeks on study, and ad hoc on the occurrence of cardiorespiratory AEs, indicated a trend towards a mean decrease in left ventricular ejection fraction at week 8 across all dose cohorts (ranging from -1.2% to -12.2%), with no obvious dose dependency. In total, four AEs of ejection fraction decreases and two AEs of left ventricular dysfunction were reported, all of which were considered at least partially treatment related.

Pharmacokinetics

Part A: dose escalation. Of the 31 patients recruited, 28 were enrolled into four sequential dose-escalating cohorts of 25 mg, 50 mg, 75 mg, and 100 mg, all twice daily.

Table 2. Patients with the most common all-causality AEs ($\geq 20\%$ across all patient groups) and dose-limiting toxicities by preferred term: safety analysis set

Adverse event	AZD6244 twice daily dose, number (%) of patients												Total no. of patients (n = 56)	
	Part A				Part B		Part A+B							
	25 mg (n = 6)		50 mg (n = 7)		75 mg (n = 7)		100 mg (n = 8)		75 mg (n = 28)		75 mg (n = 35)			
Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	
Fatigue	2 (33.3)	1 (16.7)	4 (57.1)	0	3 (42.9)	2 (28.6)*	7 (87.5)	1 (12.5)	14 (50.0)	4 (14.3)	17 (48.6)	6 (17.1)	38 (67.9)	
Acneiform dermatitis	2 (33.3)	0	6 (85.7)	0	2 (28.6)	1 (14.3)	5 (62.5)	1 (12.5) [†]	17 (60.7)	1 (3.6)	19 (54.3)	2 (5.7)	35 (62.5)	
Nausea	4 (66.7)	0	3 (42.9)	1 (14.3)	6 (85.7)	0	4 (50.0)	1 (12.5)	11 (39.3)	0	17 (48.6)	0	30 (53.6)	
Diarrhea	2 (33.3)	0	2 (28.6)	0	6 (85.7)	0	4 (50.0)	0	13 (46.4)	0	19 (54.3)	0	27 (48.2)	
Peripheral edema	1 (16.7)	0	2 (28.6)	0	5 (71.4)	0	4 (50.0)	0	11 (39.3)	1 (3.6)	16 (45.7)	1 (2.9)	24 (42.8)	
Cough	3 (50.0)	0	1 (14.3)	0	4 (57.1)	0	0	0	11 (39.3)	0	15 (42.9)	0	19 (33.9)	
Dry skin	0	0	1 (14.3)	0	3 (42.9)	0	3 (37.5)	0	12 (42.9)	0	15 (42.9)	0	19 (33.9)	
Exertional dyspnea	2 (33.3)	0	3 (42.9)	0	2 (28.6)	0	0	0	8 (28.6)	1 (3.6)	10 (28.6)	1 (2.9)	16 (28.6)	
Anorexia	2 (33.3)	0	1 (14.3)	0	0	0	2 (25.0)	0	10 (35.7)	0	10 (28.6)	0	15 (26.8)	
Constipation	1 (16.7)	0	4 (57.1)	0	2 (28.6)	0	1 (12.5)	0	7 (25.0)	0	9 (25.7)	0	15 (26.8)	
Vomiting	3 (50.0)	0	1 (14.3)	1 (14.3)	2 (28.6)	0	2 (25.0)	0	4 (14.3)	2 (7.1)	6 (17.1)	2 (5.7)	15 (26.8)	
Abdominal pain	0	0	3 (42.9)	0	3 (42.9)	0	1 (12.5)	0	5 (17.9)	0	8 (22.9)	0	12 (21.4)	
Pruritus	1 (16.7)	0	4 (57.1)	0	3 (42.9)	0	1 (12.5)	0	4 (14.3)	0	7 (20.0)	0	13 (23.2)	
Pleural effusion	0	0	0	0	0	0	0	1 (12.5) [‡]	0	0	0	0	1 (1.8)	

NOTE: Part B includes 75 mg Hyd-Sulfate capsule and 100 mg free-base suspension dosing at days 1 or 8.

*One DLT of grade 3 fatigue was reported at 75 mg twice daily in the first 22 days of dosing.

[†]One DLT of grade 3 acneiform dermatitis was reported at 100 mg twice daily in the first 22 days of dosing.

[‡]One DLT of grade 3 pleural effusion was reported at 100 mg twice daily in the first 22 days of dosing.

Table 3. Summary of pharmacokinetic parameters in Part A: (A) AZD6244. (B) *N*-desmethyl AZD6244

(A) AZD6244					
AZD6244 Hyd-Sulfate capsule dose	Summary statistic	Day 1 (single dose)			
		C_{max}	t_{max}	AUC	$t_{1/2}$
		ng/mL	h	ng×h/mL	h
25 mg	Mean*	369	1.27	1,361	6.98
	Range	268-533	1.00-2.00	1,060-1,910	5.77-8.28
	<i>n</i>	7	7	6	6
50 mg	Mean*	1,036	1	3,075	7.22
	Range	570-1,800	0.50-1.50	1,500-6,430	4.94-10.1
	<i>n</i>	7	7	3	3
75 mg	Mean*	1,207	1.5	6,335	5.33
	Range	611-2,000	1.00-2.02	5260-8510	3.87-7.44
	<i>n</i>	8	8	5	5
100 mg	Mean*	1,483	1.04	7,055	6.8
	Range	235-2,690	1.00-2.10	4,260-12,500	3.90-8.66
	<i>n</i>				
(B) <i>N</i>-desmethyl AZD6244†					
AZD6244 Hyd-Sulfate capsule dose	Summary statistic	Day 1 (single dose)			
		C_{max}	t_{max}	AUC	$t_{1/2}$
		ng/mL	h	ng×h/mL	h
75 mg	<i>n</i>	7	7	3	5
	Mean*	78.1	1.5	820	12.7
	Range	36.8-173	1.00-3.95	417-1,520	4.60-34.3
100 mg	<i>n</i>	8	8	4	4
	Mean*	97.3	1.5	506	8.86
	Range	14.3-245	1.00-8.00	374-696	5.64-10.4

Abbreviation: NC, not calculated/non calculable.

*Geometric mean for C_{max} , AUC and AUC₀₋₁₂; arithmetic mean for $t_{1/2}$, CL/F, Vss/F, median for t_{max} .

†Only the 75-mg and 100-mg cohort data are presented for *N*-desmethyl AZD6244: at 25 mg and 50 mg the concentrations were below the assay limit of quantification prior to the terminal phase for the majority of patients, therefore AUC and $t_{1/2}$ values could generally not be determined.

AZD6244 plasma pharmacokinetic parameters were similar after single (day 1) and multiple (day 8) dosing, suggesting minimal accumulation over time after twice-daily dosing, consistent with the $t_{1/2}$ observed (Table 3 and Fig. 2A; day 8 plasma concentration data not shown).

The Hyd-Sulfate capsule had a relatively rapid absorption, with a median t_{max} of ~1.0 to 1.6 hours and a mean $t_{1/2}$ of ~5 to 8 hours. CL/F and Vss/F were consistent across the dose range studied, with mean values of ~12 to 23 L/h and 87 to 126 L, respectively (Table 3).

N-desmethyl AZD6244 had a similar plasma pharmacokinetic profile to AZD6244 but lower exposures (mean ratio of *N*-desmethyl AZD6244 to AZD6244 based on C_{max} and AUC was 1.8% to 15%; Table 3).

Part B: relative bioavailability of AZD6244 Hyd-Sulfate versus the free-base suspension

Twenty-nine patients in part B were randomized and 27 patients completed the relative bioavailability assessment (Fig. 1).

Similar t_{max} and mean $t_{1/2}$ values were obtained for both formulations (Supplementary Table S2 and Fig. 2B). Mean CL/F and Vss/F were also similar when taking into account differences in relative oral bioavailability between the formulations (Supplementary Table S2). Based on AUC₀₋₂₄, the estimated oral bioavailability of the Hyd-Sulfate capsule relative to the free-base suspension was 263% (90% CI, 214-322).

As the doses used in the relative bioavailability assessment were the MTD for each formulation, a comparison

Table 3. Summary of pharmacokinetic parameters in Part A: (A) AZD6244. (B) *N*-desmethyl AZD6244 (Cont'd)

Day 1 (single dose)		Day 8 (twice-daily dose)			
CL/F	Vss/F	C _{max}	t _{max}	AUC ₀₋₁₂	CL/F
L/h	L	ng/mL	h	ng×h/mL	L/h
	<i>n</i>	6	6	5	5
18.8	126	458	1.28	1,515	16.7
13.1-23.5	85.8-219	326-678	1.00-2.00	1,310-1,870	13.3-19.0
6	6	7	7	7	7
18	125	987	1.5	3381	15.8
7.77-33.3	53.8-202	532-1,440	0.95-4.00	2,040-6,350	7.88-24.5
3	3	6	6	5	5
12.1	89.3	1,439	1.49	5,448	14.8
8.82-14.2	52.3-115	922-1,770	1.00-3.98	2,940-7,830	9.58-25.5
5	5	7	7	5	5
15.1	86.6	1,365	1.58	4,758	23.2
8.02-23.4	65.4-105	856-2,630	1.00-4.03	2,710-9,590	10.4-37.0

Day 1 (single dose)		Day 8 (twice daily dose)			
CL/F	Vss/F	C _{max}	t _{max}	AUC ₀₋₁₂	CL/F
L/h	L	ng/mL	h	ng×h/mL	L/h
NC	NC	6	6	4	NC
NC	NC	66.6	1.52	365	NC
NC	NC	20.2-115	1.00-3.98	324-412	NC
NC	NC	7	7	5	NC
NC	NC	65.3	1.58	321	NC
NC	NC	43.2-187	1.00-4.03	238-407	NC

of the MTD doses was possible. C_{max} and AUC₀₋₂₄ gls-means obtained at the MTD of the capsule (1,316 ng/mL and 4,454 ng × h/mL, respectively) were statistically significantly higher than those obtained at the MTD of the free-base suspension (523 ng/mL and 2,260 ng × h/mL, respectively). Based on C_{max} and AUC₀₋₂₄, exposure of the 75 mg Hyd-Sulfate capsule relative to the 100 mg free-base suspension were estimated to be 252% (90% CI, 182-348%) and 197% (90% CI, 161-242%), respectively. There was no clear relationship between AZD6244 plasma exposure with the 75 mg twice-daily Hyd-Sulfate capsule and toxicity in the limited cohort of patients studied.

The exposure of *N*-desmethyl AZD6244 relative to AZD6244 exposure was similar for both formulations, and in agreement with results obtained in part A (data not shown).

Pharmacokinetic/pharmacodynamic relationship (parts A and B)

Inhibition of ERK phosphorylation was observed at all doses of AZD6244, the greatest degree of inhibition

occurring at the first postdose time point (1 hour). The magnitude of inhibition of TPA-induced ERK phosphorylation in lymphocytes was related to AZD6244 plasma concentrations (Fig. 2C). A mean IC₅₀ of 352 ng/mL was estimated based on the AZD6244 plasma concentrations, with an E_{max} (maximum inhibition) of approximately 91%, indicating a potential for total inhibition. In addition, as both AZD6244 and *N*-desmethyl AZD6244 are active, both are likely to be contributing to the pharmacodynamic effect.

Antitumor activity (parts A and B)

Fifty-five patients had RECIST-evaluable tumors. One complete response was reported in a 30-year-old female patient with malignant melanoma receiving 75 mg twice daily of the Hyd-Sulfate capsule in part A, which is ongoing at two years of therapy with AZD6244 (Fig. 3). Analysis of archival tumor samples from this patient confirmed the presence of a *BRAF* V600E mutation. In the 75-mg twice-daily cohort in parts A and B, 16 of 35 patients (45.7%) had stable disease for ≥6 weeks. Ten of 55 patients (18.2%; not including the patient with a complete

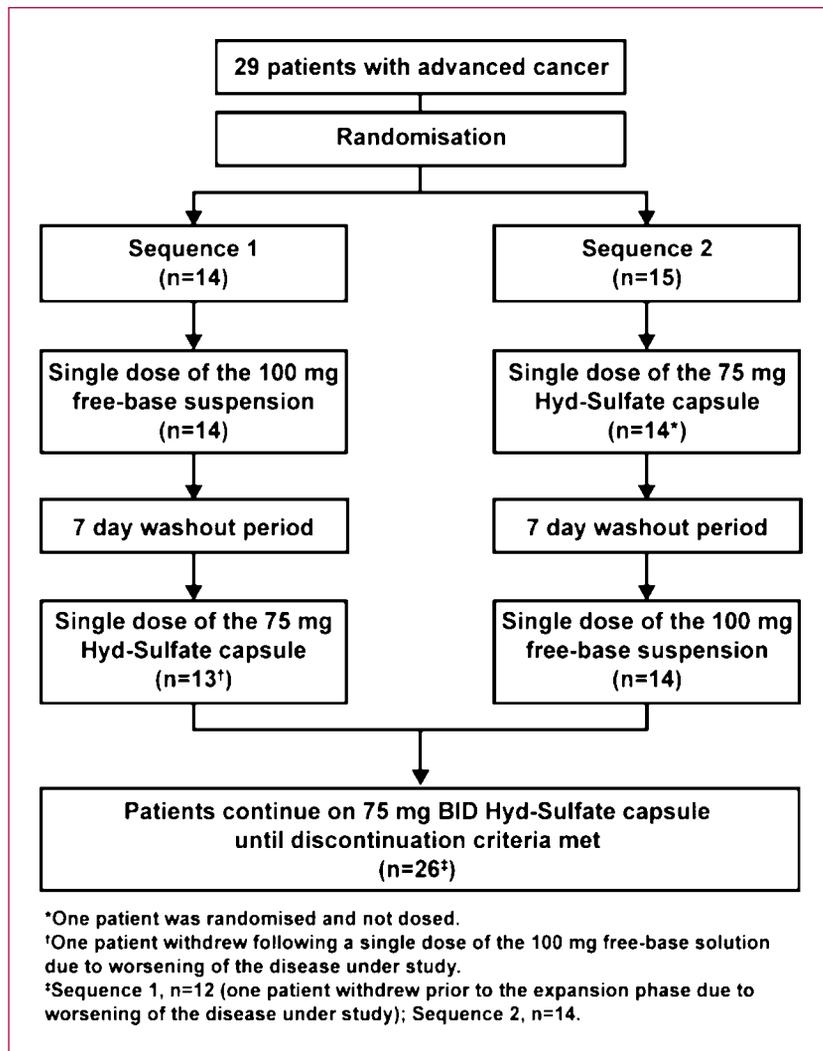


Fig. 1. Study design and patient randomization for part B: patient randomization in assessing the relative pharmacokinetic parameters of the capsule formulation versus the suspension formulation.

response) had stable disease for ≥ 16 weeks. No clear relationship was observed between antitumor activity and AZD6244 plasma exposure in the limited cohort of patients studied.

Discussion

Previous studies investigating AZD6244 used an oral free-base suspension that required oral administration of a liquid formulation twice daily (8–11). This led to substantial patient inconvenience and potentially a lack of compliance. In this first-in-human study of the AZD6244 Hyd-Sulfate oral capsule, 75 mg twice daily was established as the MTD based on two DLTs in the 100-mg twice-daily dose cohort of grade 3 dermatitis acneiform and grade 3 pleural effusion, the latter of which has not been previously reported in MEK inhibitor clinical studies, including those of the AZD6244 free-base suspension (8–14). Notably, pleural effusion occurred only once in the study, but when coupled with reports on other fluid accumula-

tion conditions (i.e., mild to moderate peripheral edema in four of eight patients at 100 mg twice daily), pleural effusion was considered a DLT. In addition to the two observed DLTs, the frequency of the most common AEs in the 100-mg twice-daily cohort (100% of patients experienced fatigue, 75% had acneiform dermatitis, 62.5% had nausea, and 50% experienced diarrhea) were also taken into account when determining the MTD of the capsule. Furthermore, as the AUC of the 75 mg twice-daily Hyd-Sulfate capsule was in excess of that reported at the MTD of the free-base suspension, increasing the dose was not considered prudent by the investigators.

Evaluation of the safety and tolerability in parts A and B showed that the safety profile of the 75 mg twice-daily Hyd-Sulfate capsule was consistent with that of the 100 mg twice-daily free-base suspension (8–11). The most common AEs at this dose were fatigue, acneiform dermatitis, nausea, and diarrhea, similar to that previously reported for the AZD6244 free-base suspension (8–11), as well as other MEK inhibitors such as PD0325901 and CI-1040

(12–15). Mild and transient visual changes (predominately blurring of vision) were reported in the present study, which is also in accordance with previous studies of the AZD6244 free-base suspension and with other MEK inhibi-

tors (8, 12–14). No significant trends in QTcF prolongation were observed in this study, although it should be noted that thorough evaluation of QTc prolongation was not a primary aim of the study. Laboratory and vital sign findings in this study were also generally consistent with those observed previously with the free-base suspension (8). Overall, the 75 mg twice-daily Hyd-Sulfate capsule was generally well tolerated, with minimal need for treatment interruptions or dose reductions. This was also shown in a case report evaluating the management of dermatologic AEs in three patients from the present study (16).

Part B of this study, which directly compared the 75 mg Hyd-Sulfate capsule with 100 mg free-base suspension, showed that the plasma exposure (C_{max} and AUC_{0-24}) for the 75 mg Hyd-Sulfate capsule was statistically significantly higher than that for the 100 mg free-base suspension. Based on AUC_{0-24} , the exposure of the 75 mg Hyd-Sulfate capsule relative to the 100 mg free-base suspension was 197% (90% CI, 161-242%). The estimated oral bioavailability of the Hyd-Sulfate capsule relative to the free-base suspension based on dose normalized AUC_{0-24} was 263% (90% CI, 241-322%). AZD6244 Hyd-Sulfate plasma pharmacokinetic parameters were in agreement across the 25 to 100 mg range studied and were similar after single and multiple dosing, suggesting minimal accumulation over time after twice-daily dosing. *N*-desmethyl AZD6244, an active circulating metabolite, had a similar plasma pharmacokinetic profile to AZD6244 but lower exposures.

The AZD6244 free-base suspension has previously been shown to reduce pERK expression in tumor biopsies and in peripheral blood mononuclear cells of treated patients (8). ERK phosphorylation levels in peripheral blood mononuclear cells, or more specifically in the lymphocyte population as shown here, may thus be considered a surrogate pharmacodynamic biomarker for MEK inhibitor activity *in vivo*. In the present study, dosing with the Hyd-Sulfate capsule caused a time-dependent suppression of pERK, with the magnitude of suppression being generally related to drug plasma concentrations; however, further work is needed to understand the relevance of this relationship to the clinical situation. As both AZD6244 and *N*-desmethyl AZD6244 are active, and likely to be contributing to the pharmacodynamic effect, a more rigorous approach may be to examine varying concentrations of both AZD6244 and *N*-desmethyl AZD6244, as opposed to considering either active moiety in isolation.

The best clinical outcome in this study was a prolonged complete response in a patient with malignant melanoma harbouring a V600E *BRAF* mutation receiving the 75 mg twice-daily Hyd-Sulfate capsule. This patient was pretreated with dacarbazine and had progressive disease after six cycles, and, following 15 months of treatment with 75 mg twice-daily AZD6244 Hyd-Sulfate capsule, the patient remained in complete response. Prolonged stable disease (≥ 16 weeks) was observed in 10 of 55 patients in parts A and B.

The free-base suspension of AZD6244 has previously been evaluated in randomized phase II trials in patients with advanced/metastatic melanoma, colorectal cancer,

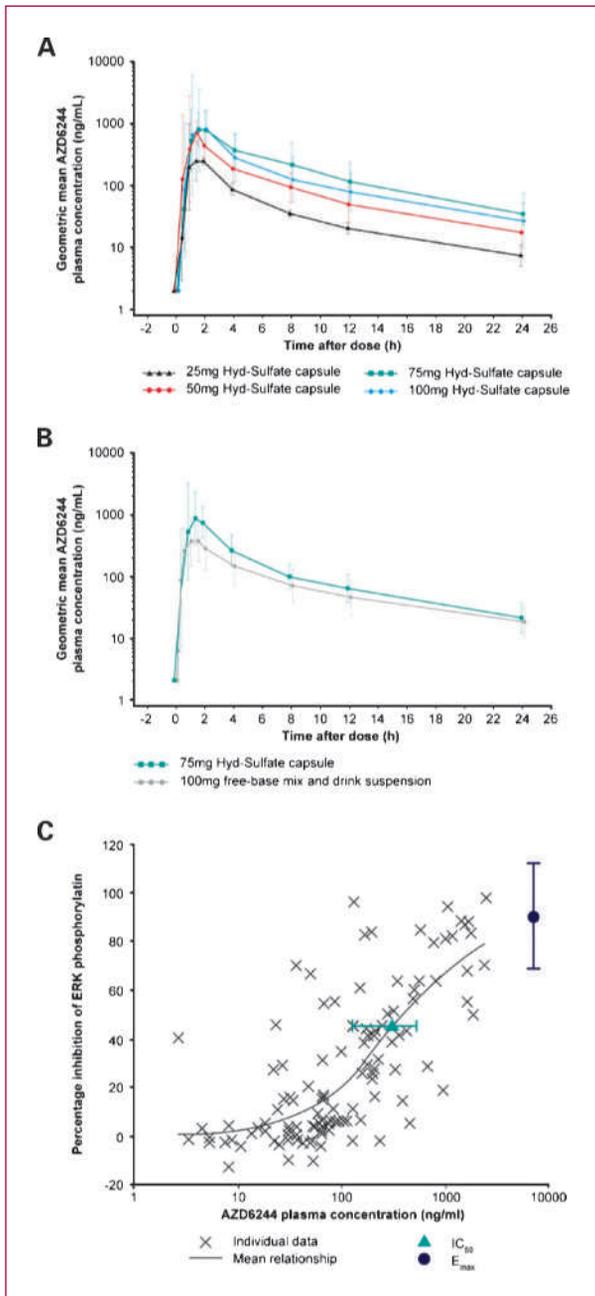


Fig. 2. Pharmacokinetic and pharmacodynamic analyses of AZD6244 Hyd-Sulfate. Geometric mean (\pm SD) AZD6244 plasma concentrations (ng/mL) versus time following single doses of (A) 25 to 100 mg Hyd-Sulfate capsule in part A, and (B) 75 mg Hyd-Sulfate capsule and 100 mg free-base suspension in part B, on a log-linear scale. C, inhibition of ERK phosphorylation in *ex-vivo* TPA-treated peripheral blood lymphocytes versus AZD6244 plasma concentration in parts A and B. Individual points represent individual patient values. Results are from blood samples collected 1, 4, 8, and 24 h postdose on days 1 (parts A and B) and 8 (part B only).

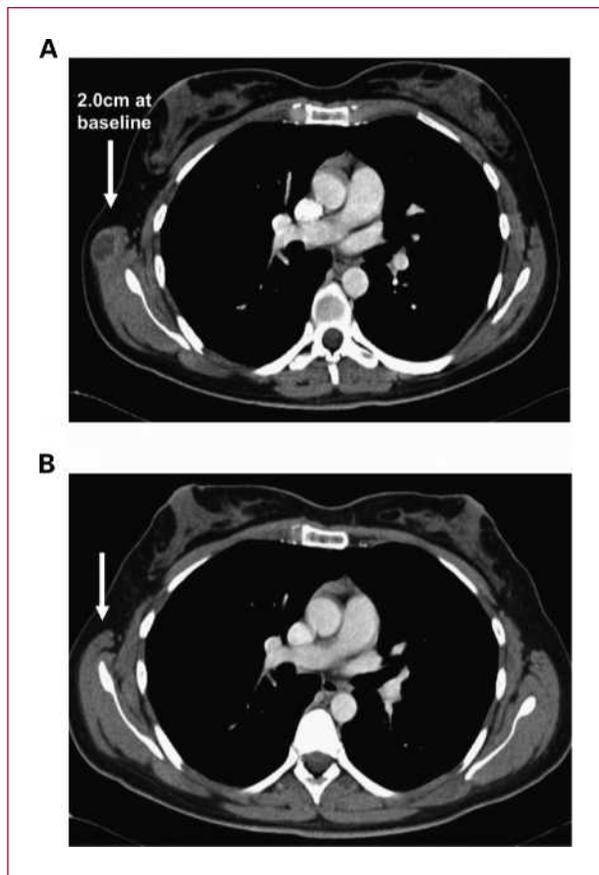


Fig. 3. BRAF mutation-positive melanoma patient with a prolonged complete response after 16 wk therapy. A, image taken July 24, 2007. B, image taken November 7, 2007.

and NSCLC (9–11). AZD6244 did not show superiority to the standard of care comparators in these studies; however, no significant differences were observed between AZD6244 and its comparators for the primary end point of each study (progression free survival in the melanoma study, and disease progression event count in the colorectal cancer and NSCLC studies). Furthermore, partial responses following treatment with AZD6244 were reported for six patients in the melanoma study and two

patients in the NSCLC study (9, 10). Given the increased exposure of the Hyd-Sulfate capsule relative to the free-base suspension, this new formulation potentially offers increased benefit for patients over that previously reported. AZD6244 Hyd-Sulfate is also currently being evaluated in combination with docetaxel in *KRAS* mutation positive NSCLC patients (ClinicalTrials.gov number, NCT00890825), and in combination with dacarbazine in patients with melanoma bearing *BRAF* mutations (ClinicalTrials.gov number, NCT00936221) to assess whether such regimens will further improve AZD6244 antitumor activity.

In conclusion, this study establishes that the 75 mg twice-daily Hyd-Sulfate capsule of the MEK1/2 inhibitor AZD6244 has a manageable safety and tolerability profile. Together with the favorable pharmacokinetic and pharmacodynamic profile and the prolonged anticancer activity observed with the AZD6244 Hyd-Sulfate capsule, this formulation should be evaluated in phase II trials, and patients with tumors bearing the *BRAF* V600 E mutation should be considered as suitable candidates. This formulation should also be taken forward in combination phase I trials of AZD6244 with conventional cytotoxic agents or other targeted therapies.

Disclosure of Potential Conflicts of Interest

K. Brown, M. Cantarini, C. Morris, P. Smith, S. George, AstraZeneca employees, AstraZeneca shareholdings; S.G. Eckhardt, K. Lewis, AstraZeneca commercial research grant; S. Kaye, AstraZeneca clinical advisory board. All other authors report no conflicts of interest.

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The First-in-Human Study of the Hydrogen Sulfate (Hyd-Sulfate) Capsule of the MEK1/2 Inhibitor AZD6244 (ARRY-142886): A Phase I Open-Label Multicenter Trial in Patients with Advanced Cancer

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