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A phase I dose-finding, safety and tolerability study of AZD8330 in patients with advanced malignancies

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Maximum tolerated dose

Abstract ***Objective:*** This is the first clinical study of the MEK1/2 inhibitor AZD8330 (ARRY-424704). This phase I study defined the maximum tolerated dose (MTD) and assessed the safety, tolerability, pharmacokinetics and pharmacodynamics of AZD8330 in patients with advanced malignancies.

Methods: Patients with refractory cancer or cancer with no standard therapy received either once-daily (OD) or twice-daily (BID) oral AZD8330 on day 1 followed by a 7-day washout period and continuous dosing from day 8. The starting dose was 0.5 mg with dose escalations in subsequent cohorts until a non-tolerated dose was reached.

Results: Eighty-two patients received AZD8330 across 11 cohorts. The most frequent AZD8330-related adverse events were acneiform dermatitis (13/82, 16%), fatigue (11/82, 13%), diarrhoea (11/82, 13%) and vomiting (9/82, 11%). Four patients experienced dose-limiting toxicities: mental status changes (40 mg OD; 2/9 patients and 60 mg OD; 1/3) and rash (20 mg BID; 1/9). The MTD was defined as 20 mg BID. AZD8330 exposure increased approximately proportionally with dose across the dose range 0.5–60 mg OD. Dose-dependent modulation of phosphorylated ERK in peripheral blood mononuclear cells (PBMCs) was observed at doses $\geqslant 3$ mg. One patient had a partial response and thirty-two (39%) had stable disease, with a duration >3 months in 22 patients, assessed by Response Evaluation Criteria in Solid Tumors.

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Conclusion: AZD8330 has a manageable toxicity profile at the MTD of 20 mg BID, and target inhibition was confirmed in PBMCs. One patient with malignant melanoma had a partial response.

1. Introduction

MAPK ERK kinase (MEK) 1 and 2 are essential enzymes in the Ras/Raf/MEK/ERK protein kinase pathway that regulate cellular proliferation. Deregulation of this pathway occurs frequently in a variety of tumours and promotes cell survival and tumour growth. The only known substrates of MEK1/2 are the extracellular signal-regulated kinases ERK1 and ERK2; MEK inhibition is therefore an attractive therapeutic target in cancer.

Several MEK inhibitors are currently being investigated clinically in various tumour types.[1–5] AZD8330 (ARRY-424704) is a potent, selective, uncompetitive MEK1/2 inhibitor, with a half-maximum inhibitory concentration (IC_{50}) of 7 nM.[6] In tumour xenograft models, AZD8330 demonstrated dose-dependent tumour growth inhibition of approximately 90% at tolerated doses (1.0 mg/kg once daily [OD]).[6] These preclinical results suggest that AZD8330 may provide effective tumour growth inhibition with a favourable tolerability profile.

This is the first clinical study of AZD8330, designed to determine its safety, tolerability, pharmacokinetic (PK) and pharmacodynamic (PD) profile in patients with advanced malignancies.

2. Patients and methods

2.1. Patients

Eligible patients were $\geqslant$18 years of age, with a confirmed diagnosis of cancer refractory to standard therapy or for which no standard therapy exists. Other inclusion criteria included a World Health Organization performance status of 0–2 and adequate renal, hepatic and cardiac function.

2.2. Trial design

This was an open-label, phase I dose-escalation study conducted in two centres in the USA and one centre in Norway (NCT00454090). Enrolment was from March 2007 to April 2010. All patients provided written informed consent and the study was conducted in accordance with the Declaration of Helsinki.

2.3. Interventions

Eligible patients in each cohort (of six to nine evaluable patients) received either an OD or twice-daily (BID) dose of AZD8330 (AstraZeneca, Alderley Park, Cheshire, UK) on day 1 followed by a 7-day washout period and continuous OD or BID dosing, respectively, from day 8. Patients were not permitted food 2 h preceding and 1 h following dosing. The starting dose of AZD8330 was 0.5 mg; subsequent cohorts received an increased dose of AZD8330 determined by the Safety Review Committee (SRC), until a non-tolerated dose was reached. A non-tolerated dose was defined as the dose at which $\geqslant$2/6 patients in a cohort experienced a dose-limiting toxicity (DLT) within 35 days of commencing treatment. Patients remained on treatment until disease progression and for as long as they continued to derive clinical benefit. All patients were followed until withdrawal of consent or the end of the study (6 months following first treatment dose).

2.4. End-points

The primary objective of the study was to assess the safety and tolerability of AZD8330. Secondary objectives were: to determine the PK profile of AZD8330 following both single and multiple dosing, to investigate the effect of AZD8330 on pERK in peripheral blood mononuclear cells (PBMCs) and to explore relationships between PK and PD parameters. Assessment of AZD8330 efficacy was an exploratory end-point.

2.5. Safety

All adverse events (AEs) were recorded from the time of informed consent until 30 days after study treatment was discontinued, using Common Terminology Criteria for Adverse Events (CTCAE) version 3. General safety monitoring information is provided in the Supplementary section. AEs were followed to resolution where possible.

A DLT was defined as any CTCAE grade $\geqslant$3 AE unrelated to underlying disease; continuous dose interruption for >2 weeks for any toxicity considered to be possibly related to AZD8330; or any toxicity considered to be clinically significant by the investigator, within 35 days of first dose. The maximum tolerated dose (MTD) was defined as the last dose tested below the non-tolerated dose in six evaluable patients.

2.6. PK analysis

Serial venous blood samples (2 mL) were taken predose, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10 (for 0.5–1.5 mg only),

12 (pre-second dose for BID) and 24 h post-dose (for day 15 taken prior to day 16 dose) on days 1 and 15 and 48 h post-dose (day 1 only, 3–60 mg). Sparse sampling was performed on days 22 and 29 at pre-dose, 0.5, 1, 2 and 4 h post-dose.

The PK parameters were derived using non-compartmental methods with WinNonlin® Professional version 5.2 (Pharsight Corp., Mountain View, California). Additional PK methods are provided in the Supplementary section.

2.7. PD analysis

Blood samples (2 × 2 mL) were taken pre-dose, 1.5, 4, 8 and 24 h post-dose on day 1 for pERK analysis in PBMCs. A flow cytometric immunoassay was used to measure phorbol ester stimulated pERK production in lymphocytes.

2.8. PK/PD relationship

The relationship between AZD8330 plasma concentrations and inhibition effects was investigated using the maximum inhibition effect (E_{max}) model.

2.9. Antitumour activity

Tumour response was assessed every 8 weeks using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0.

2.10. Statistical methods

No formal statistical analysis was performed; data were summarised using descriptive statistics and graphical presentations.

3. Results

3.1. Patients

Eighty-two patients were enrolled in 11 cohorts: AZD8330 OD 0.5 mg ($n = 9$), 0.75 mg ($n = 6$), 1.5 mg ($n = 6$), 3 mg ($n = 9$), 6 mg ($n = 7$), 12 mg ($n = 8$), 12 mg ($n = 7$; repeated cohort), 20 mg ($n = 9$), 40 mg ($n = 9$), 60 mg ($n = 3$) and AZD8330 BID 20 mg ($n = 9$) (Table 1).

Table 1
Summary of patient characteristics.

Baseline characteristics, *n* (%)		*n* (%)
Gender[a]	Female	43 (52)
	Male	40 (48)
Age (years)[a]	>16–<65	55 (66)
	⩾65	28 (34)
WHO performance status	0	29 (35)
	1	50 (61)
	2	3 (4)
Primary tumour location[a]	Colorectal	24 (29)
	Skin/soft tissue	20 (24)
	(Melanoma)	(18; 22)
	Other	13 (16)
	Head and neck	8 (10)
	(Neurofibromatosis, type 2)	(1; 1)
	(Trachea)	(1; 1)
	(Right parotid gland)	(1; 1)
	(Metastatic acinic carcinoma)	(1; 1)
	(Adenoid cystic carcinoma)	(2; 2)
	(Metastatic squamous cell of larynx)	(1; 1)
	Lung	4 (5)
	(Non-small cell lung cancer)	(2; 2)
	Thyroid	3 (4)
	(Papillary)	(1; 1)
	Bladder	2 (2)
	Pancreas	2 (2)
	Prostate	2 (2)
	Unknown	1 (1)
	Oesophagus	1 (1)
	Ovary	1 (1)
	Uterus	1 (1)
	Cervix	1 (1)
Prior treatment	Radiotherapy	47 (57)
	Chemotherapy:	
	Prior regimens	
	0	7 (9)
	1–2	21 (26)
	3–5	54 (66)
	>5	0 (0)

WHO, World Health Organization.
[a] $n = 83$, one patient did not receive treatment.

3.2. Safety

AEs were reported by 77/82 patients (94%) (Table 2). The most frequent grade ⩾3 AEs were pneumonia ($n = 3$), left ventricular (LV) dysfunction ($n = 3$), hallucination ($n = 3$), respiratory failure ($n = 3$) and hypotension ($n = 3$) (Supplementary Table). In total, 47/82 (57%) patients had an AE considered to be AZD8330-related by the investigator, with the most frequent being acneiform dermatitis (13/82, 16%), fatigue (11/82, 13%), diarrhoea (11/82, 13%) and vomiting (9/82, 11%). Only LV dysfunction was considered related to AZD8330 by the investigator. Three patients experienced LV ejection dysfunction, of which one was a serious adverse event (SAE) deemed AZD8330 treatment related. All cases resolved. The SRC elected to expand the 12 mg OD cohort because of the inconsistent reports of reduced ejection fraction and the multigated acquisition scans were included as a protocol amendment. No further results of concern were observed in the expanded cohort, including electrocardiographic parameters or laboratory abnormalities of troponin and B-type natriuretic peptide (BNP). Thus, 12 mg OD was declared tolerable.

Table 2
Summary of adverse events (⩾5% in total) regardless of causality by preferred term.

Preferred term, *n* (%)	Total ($n = 82$)		0.5 mg OD	0.75 mg OD	1.5 mg OD	3 mg OD	6 mg OD	12 mg OD	20 mg OD	40 mg OD	60 mg OD	20 mg BID
	Any grade	Grade ⩾3	($n = 9$)	($n = 6$)	($n = 6$)	($n = 9$)	($n = 7$)	($n = 15$)	($n = 9$)	($n = 9$)	($n = 3$)	($n = 9$)
Fatigue	29 (35)	2 (3)	3 (33)	0 (0)	4 (67)	2 (22)	0 (0)	4 (27)	5 (56)	7 (78)	1 (33)	3 (33)
Diarrhoea	21 (26)	1 (1)	4 (44)	0 (0)	0 (0)	0 (0)	0 (0)	6 (40)	0 (0)	4 (44)	1 (33)	6 (67)
Vomiting	19 (23)	1 (1)	3 (33)	0 (0)	2 (33)	0 (0)	2 (29)	4 (27)	0 (0)	4 (44)	1 (33)	3 (33)
Constipation	16 (20)	0 (0)	6 (67)	0 (0)	2 (33)	0 (0)	3 (43)	0 (0)	2 (22)	0 (0)	0 (0)	3 (33)
Decreased appetite	15 (18)	0 (0)	4 (44)	0 (0)	0 (0)	0 (0)	0 (0)	3 (20)	4 (44)	0 (0)	1 (33)	3 (33)
Nausea	15 (18)	1 (1)	5 (56)	0 (0)	0 (0)	1 (11)	0 (0)	2 (13)	0 (0)	3 (33)	0 (0)	4 (44)
Peripheral oedema	15 (18)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (29)	2 (13)	2 (22)	5 (56)	1 (33)	3 (33)
Urinary tract infection	15 (18)	2 (3)[a]	2 (22)	2 (33)	1 (17)[a]	2 (22)	0 (0)	3 (20)	2 (22)	3 (33)	0 (0)	0 (0)
Acneiform dermatitis	13 (16)	0 (0)	1 (11)	0 (0)	0 (0)	0 (0)	0 (0)	3 (20)	3 (33)	3 (33)	0 (0)	3 (33)
Back pain	11 (13)	2 (3)	2 (22)	0 (0)	0 (0)	0 (0)	0 (0)	2 (13)	3 (33)	3 (33)	0 (0)	1 (11)
Exertional dyspnoea	11 (13)	2 (3)	3 (33)	1 (17)	2 (33)	0 (0)	0 (0)	3 (20)	0 (0)	2 (22)	0 (0)	0 (0)
Abdominal pain	9 (11)	2 (3)	2 (22)	0 (0)	0 (0)	2 (22)	0 (0)	0 (0)	2 (22)	3 (33)	0 (0)	0 (0)
Cough	7 (9)	0 (0)	0 (0)	2 (33)	3 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (22)
Dizziness	7 (9)	0 (0)	2 (22)	0 (0)	0 (0)	0 (0)	0 (0)	4 (27)	0 (0)	0 (0)	1 (33)	0 (0)
Hypomagnesaemia	7 (9)	0 (0)	4 (44)	0 (0)	0 (0)	0 (0)	1 (14)	0 (0)	2 (22)	0 (0)	0 (0)	0 (0)
Myalgia	5 (6)	0 (0)	0 (0)	1 (17)	0 (0)	0 (0)	2 (29)	0 (0)	0 (0)	2 (22)	0 (0)	0 (0)

OD, once daily; BID, twice daily.
[a] Includes *Escherichia* urinary tract infection.

Table 3
Summary of pharmacokinetic parameters.

PK parameter Mean ± SD (range)	Cohort									
Single dose (Day 1)	0.5 mg OD ($n = 9$)	0.75 mg OD ($n = 6$)	1.5 mg OD ($n = 6$)	3 mg OD ($n = 9$)	6 mg OD ($n = 7$)	12 mg OD ($n = 15$)	20 mg OD ($n = 9$)	40 mg OD ($n = 9$)	60 mg OD ($n = 3$)	20 mg BID ($n = 9$)
C_{max} (ng/mL)	0.9 ± 0.7 (0.1–2.4)	2.5 ± 0.4 (1.9–3.2)	3.2 ± 1.2 (1.8–4.8)	15.4 ± 12.1 (4.6–41.5)	15.1 ± 8.0 (3.7–25.9)	44.7 ± 24.7 (16.4–91.8)	113.3 ± 97.6 (27.5–306.0)	144.2 ± 66.3 (83.5–298.0)	160.0 ± 74.0 (110.0–245.0)	69.3 ± 41.8 (18.3–117.0)
t_{max} (h)[a]	0.5 (0.3–4.0)	0.5 (0.3–1.0)	0.5 (0.5–1.5)	0.3 (0.3–0.5)	0.5 (0.3–2.0)	0.5 (0.3–2.0)	0.5 (0.3–1.5)	0.5 (0.5–2.0)	1.0 (0.5–1.5)	1.0 (0.5–2.0)
$AUC_{0-\infty}$ (ng h/mL)	2.1 ± 0.3 (1.7–2.5)	4.2 ± 1.7 (1.9–5.8)	6.6 ± 1.5 (4.9–8.8)	17.4 ± 7.8 (4.4–29.8)	23.7 ± 5.7 (16.6–29.9)	86.3 ± 61.4 (25.8–227.9)	176.8 ± 120.5 (67.1–390.8)	708.6 ± 1095.4 (204.9–3594.1)	329.8 ± 38.2 (292.7–368.9)	186.0 ± 88.3 (132.8–316.9)
$AUC_{(0-24)}$ (ng h/mL)[b]	2.4 (1.5–3.4)[c]	4.2 ± 0.9 (3.4–5.4)	5.9 ± 1.1 (4.6–7.8)	16.9 ± 6.3 (9.0–28.1)	23.8 ± 9.8 (13.5–43.8)	80.4 ± 50.5 (25.8–200.0)	161.2 ± 114.5 (58.4–345.2)	673.1 ± 1093.8 (178.2–3562.9)	300.3 ± 42.0 (266.6–347.3)	161.0 ± 66.9 (117.1–307.9)
$t_{1/2}$ (h)	6.1 ± 1.8 (4.2–8.0)	9.0 ± 3.2 (5.0–12.9)	10.0 ± 2.3 (7.9–13.8)	14.6 ± 5.9 (7.0–25.0)	18.0 ± 8.3 (10.1–29.1)	12.9 ± 4.2 (5.5–19.7)	9.6 ± 3.0 (5.7–13.3)	12.2 ± 4.4 (5.3–17.6)	14.7 ± 2.7 (11.6–16.9)	3.8 ± 1.1 (2.6–4.8)
CL/F (L/h)	245.7 ± 37.6 (198.1–292.8)	215.4 ± 124.9 (129.0–400.0)	236.8 ± 53.4 (170.7–303.5)	233.5 ± 187.2 (100.8–677.0)	266.3 ± 66.5 (200.8–362.2)	203.7 ± 123.0 52.7–464.6)	165.3 ± 92.2 (51.2–297.9)	121.7 ± 61.1 (11.1–195.2)	183.6 ± 21.2 (162.7–205.0)	122.0 ± 41.2 (63.1–150.6)
V_Z/F (L)	2196.5 ± 844.4 (1201.2–3394.8)	2434.0 ± 590.1 (1593.2–2872.3)	3339.5 ± 658.1 (2453.6–4138.8)	4300.9 ± 2019.5 (1549.7–6821.1)	6420.1 ± 1777.5 (4081.7–8617.1)	3583.8 ± 2385.1 (1275.9–9032.8)	2548.8 ± 1821.8 (420.5–5137.5)	2404.8 ± 1596.4 (85.7–4553.8)	3855.8 ± 533.0 (3440.0–4456.6)	695.8 ± 338.2 (238.1–1021.3)
Multiple dosing (Day 15)	0.5 mg OD ($n = 9$)	0.75 mg OD ($n = 6$)	1.5 mg OD ($n = 6$)	3 mg OD ($n = 9$)	6 mg OD ($n = 7$)	12 mg OD ($n = 15$)	20 mg OD ($n = 11$)	40 mg OD ($n = 9$)	60 mg OD ($n = 3$)	20 mg BID ($n = 7$)
C_{max} (ng/mL)	1.0 ± 0.9 (0.2–2.9)	2.0 ± 1.1 (0.7–3.6)	3.7 ± 2.1 (0.7–6.2)	14.7 ± 7.3 (2.7–22.3)	24.1 ± 17.4 (10.8–60.9)	45.8 ± 18.2 (14.3–72.9)	116.9 ± 101.0 (23.5–281.0)	119.1 ± 60.7 (30.7–213.0)	160.0 ± 113.1 (56.9–281.0)	113.5 ± 94.0 (32.6–319.0)
t_{max} (h)[a]	0.5 (0.3–1.5)	0.8 (0.3–1.5)	0.5 (0.3–1.0)	0.5 (0.3–0.5)	0.5 (0.3–0.5)	0.8 (0.3–2.0)	0.5 (0.5–6.0)	1.0 (0.5–1.5)	1.5 (0.5–2.0)	1.0 (0.5–6.0)
$AUC_{(0-24)}$ (ng h/mL)[b]	2.9 (2.4–3.4)[c]	5.1 ± 1.8 (2.8–7.0)	9.3 ± 4.1 (4.0–13.2)	17.7 ± 10.1 (5.2–29.9)	29.8 ± 6.3 (23.3–35.6)	80.0 ± 33.3 (40.8–155.0)	181.5 ± 101.2 (90.7–324.2)	368.9 ± 207.9 (199.5–762.2)	351.1 (319.7–382.5)[c]	252.9 ± 170.0 (121.0–502.1)
R_{AC}	-	1.4 ± 0.4 (1.1–1.9)	1.5 ± 0.6 (0.7–2.1)	1.2 ± 0.2 (1.1–1.5)	1.3 ± 0.2 (1.1–1.6)	1.5 ± 0.4 (1.0–2.1)	1.1 ± 0.3 (0.7–1.4)	1.3 ± 0.3 (0.9–1.9)	1.2 (1.1–1.2)[c]	1.4 ± 0.3 (1.0–1.6)
CL/F (L/h)	242.8 ± 147.2 (98.9–537.8)	166.4 ± 66.9 (106.5–264.6)	187.4 ± 107.2 (113.8–373.7)	210.8 ± 177.7 (66.8–574.8)	187.5 ± 65.0 (68.4–257.3)	159.3 ± 60.9 (75.1–294.5)	161.7 ± 85.9 (43.8–284.8)	150.2 ± 69.4 (52.5–260.1)	173.6 ± 15.6 (156.9–187.7)	103.0 ± 51.5 (39.8–165.3)
Temporal parameter change[e]	1.1[d]	1.3 ± 0.4 (1.1–1.7)	1.2 ± 0.6 (0.6–1.7)	1.1 ± 0.1 (0.9–1.2)	1.0 ± 0.2 (0.9–1.2)	1.5 ± 0.4 (0.8–2.0)	1.0 ± 0.3 (0.6–1.3)	1.2 ± 0.3 (0.8–1.7)	1.1 (1.0–1.1)[c]	1.3 ± 0.3 (0.9–1.6)

PK, pharmacokinetic; SD, standard deviation; BID, twice daily; OD, once daily; $AUC_{0-\infty}$, area under the plasma–concentration curve from zero to infinity; AUC_{0-12}, area under the plasma–concentration curve from zero to 12 h post-dose; AUC_{0-24}, area under the plasma–concentration curve from zero to 24 h post-dose; C_{max}, maximum plasma concentration; t_{max}, time to maximum plasma concentration; $t_{1/2}$, half-life; CL/F, oral clearance from plasma; V_z/F, apparent steady-state volume of distribution; R_{AC}, accumulation ratio based on $AUC_{(0-24)}$ day 15/$AUC_{(0-24)}$ day 1 for OD dosing or $AUC_{(0-12)}$ day 15/$AUC_{(0-12)}$ on day 1 for BID dosing.

[a] Median (range).

[b] For BID Dosing AUC_{0-12} is shown.

[c] $n = 2$.

[d] $n = 1$.

[e] Temporal parameter change was calculated as $AUC_{(0-24)}$ on day 15/$AUC_{0-\infty}$ on day 1 for OD dosing, and $AUC_{(0-12)}$ on day 15/$AUC_{0-\infty}$ on day 1 for BID dosing.

SAEs were reported in all cohorts (32/82 patients; 39%) except the 0.75 mg OD cohort; the majority were not considered AZD8330 related by the investigator. Additional safety results are provided in the Supplementary section. One instance of each of the following AZD8330-related SAEs was recorded: 12 mg OD, LV dysfunction; 40 mg OD, unilateral visual hallucination, hallucination, vomiting, worsening diarrhoea and neutropenic fever; 60 mg OD, mental status change; 20 mg BID, folliculitis, rhabdomyolysis and hypoxia. In total, four DLTs were observed during the study. Three patients, in the 40 mg (2/9 patients) and the 60 mg (1/3) OD cohorts, experienced mental status changes including: confusion, mental status change and hallucinations. These DLTs occurred 27, 46 and 48 days after the first dose of AZD8330 and were reversible upon treatment cessation. The mental status AEs seen in the 40 mg OD cohort occurred outside the 30-day DLT evaluation period, allowing per protocol dose escalation to 60 mg OD. Mental status AEs were also seen in the 60 mg OD cohort. No relationship between mental status change AEs and exposure was seen, as measured by the maximum observed plasma concentration (C_{max}) and the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$), on days 1 and 15. The AZD8330 40 mg and 60 mg doses were defined as non-tolerated. A 20 mg BID dose was then assessed as an interim dose to minimise the mental status change AEs observed in the 40 and the 60 mg cohorts. One of nine patients in the 20 mg BID cohort experienced a grade 3 rash 19 days after starting AZD8330, which resolved after a 21-day treatment interruption. Thus, AZD8330 20 mg BID met the definition of the MTD.

Ten patients permanently discontinued treatment because of an AE. The following AEs leading to discontinuation were considered to be related to AZD8330 by the investigator: hallucination, intestinal obstruction, pathological fracture, pneumonia, increased blood bilirubin and pancreatitis. Four patients in the 40 mg OD cohort and one patient in the 20 mg OD cohort had a dose reduction. Ten patients died between days 13 and 57 of study treatment, all due to disease progression.

3.3. PK analysis

A summary of PK parameters is displayed in Table 3. Steady-state plasma concentrations are shown in Fig. 1. Following a single dose (0.5–60 mg OD), AZD8330 was rapidly absorbed and the median time to maximum plasma concentration (t_{max}) ranged from 0.3 to 1 h (Table 3). The terminal elimination half-life ($t_{1/2}$; Table 3), oral clearance and volume of distribution appeared to be independent of dose. Steady state was achieved by day 15 (after 8 days of multiple dosing); AZD8330 exposure (AUC from 0 to 4 h post-dose) was comparable on days 15, 22 and 29 (data not shown). AZD8330 accumulation (R_{AC} in Table 3) was minimal with no time-dependent PK changes observed following multiple dosing; the temporal change parameter was approximately 1. Exposure to AZD8330 (C_{max} and $AUC_{0-\infty}$) increased approximately proportionally with dose across the dose range 0.5–60 mg OD.

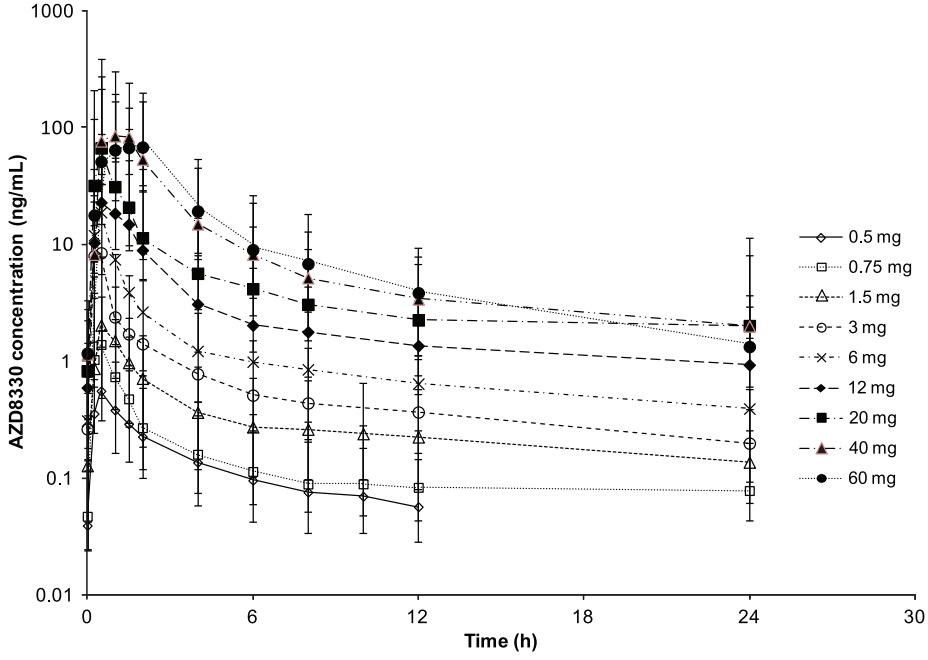

Fig. 1. Geometric mean ± SD plasma concentrations of AZD8330 versus time following multiple dosing.

Table 4
Percentage of pERK in fixed peripheral blood mononuclear cells post-AZD8330 dose.

	pERK normalised to pre-dose (%)[a] Gmean (95% CI)								
Time point (Day 1) (h)	0.5 mg OD ($n = 8$)	0.75 mg OD ($n = 3$)	1.5 mg OD ($n = 6$)	3 mg OD ($n = 7$)	6 mg OD ($n = 7$)	12 mg OD ($n = 13$)	20 mg OD ($n = 9$)	40 mg OD ($n = 7$)	60 mg OD ($n = 3$)	20 mg BID ($n = 9$)
0.5	ND	ND	ND	58 (33;101)	47 (25;86)	30 (15;61)	14 (4;50)	5 (2;15)	22 (7;67)	22 (9;53)
1.5	98 (91;106)	101 (71;145)	99 (92;105)	82 (61;110)	48 (28;84)	37 (20;69)	24 (12;47)	4 (1;13)	11 (2;75)	25 (10;63)
4	92 (78;109)	92[b]	104 (97;111)	87 (72;105)	82 (65;103)	87 (70;107)	82 (75;90)	29 (11;78)	13 (0;395)	67 (49;92)
8	102 (98;105)	101[b]	104 (97;112)	96 (89;104)	84 (63;112)	98 (85;113)	93 (85;100)	33 (9;115)	34 (1;782)	70 (51;97)
24	99 (95;103)	ND	106 (94;120)	97 (84;112)	80 (64;101)	96 (83;112)	93 (86;100)	62 (41;94)	65 (24;174)	66 (40;110)

OD, once daily; BID, twice daily; ND, no data.
[a] Pre-dose pERK equals 100%.
[b] $n = 1$.

3.4. PD analysis

Dose- and time-dependent modulation of pERK in PBMCs was observed at doses $\geqslant 3$ mg (Table 4). At 20 mg BID, pERK was reduced to an average of 25% of pre-dose levels after 1.5 h post-dose and remained at an average of 66% after 12 h post-second dose.

3.5. PK/PD relationship

The E_{max} of AZD8330 was 97.6% (% coefficient of variation [CV] 3.6) (Fig. 2), with the AZD8330 plasma concentration that gave half the E_{max} being 10.1 ng/mL (%CV 11.05) or 2 nM free plasma AZD8330. AZD8330 *in vitro* inhibits MEK1/2 with an IC_{50} of 7 nM,[6] comparable to the EC_{50} (2 nM) observed in this study. The PK/PD model predicted that following AZD8330 20 mg BID administration to steady state, the population median inhibition of pERK would be approximately 80% at the time corresponding to C_{max} and at 4 h post-dose the percentage inhibition of pERK would decline to approximately 40%.

3.6. Antitumour activity

One patient in the 40 mg OD cohort with malignant melanoma achieved a partial RECIST response. Thirty-two patients (39%) with a wide range of tumour types had stable disease, with a duration >3 months in 22 patients. One patient with *BRAF*-positive papillary thyroid cancer had stable disease for 1 year, and one patient with *BRAF*-positive melanoma had stable disease for 6 months. Molecular tumour characterisation was not a formal part of the study protocol.

4. Discussion

This large phase I study presents the first clinical results of the MEK1/2 inihibitor AZD8330, and demonstrates a manageable toxicity profile for AZD8330 at the MTD of 20 mg BID. Although AZD8330 was verified as a MEK inhibitor through confirmed inhibition of pERK in this study, its toxicity profile diverged somewhat from those of other known MEK inhibitors.

The most frequent AZD8330-related toxicities of acneiform dermatitis, fatigue, diarrhoea and vomiting in this study are consistent with those seen with other MEK inhibitors, although they occurred at a lower rate. Rash-related toxicity is the most common AE reported with MEK inhibitors (74–92%),[1,3,5] but limited rash-related toxicity was observed with AZD8330 (28%), with one rash DLT at 20 mg BID. Eye-related toxicity is often reported with MEK inhibitors (12–23%),[1,3,7,8] but did not occur as often in this study (4%) and no instances of central serous retinopathy or retinal vein occlusion[3] were observed. Cardiac events have been previously reported with other MEK inhibitors[3,5] and thus

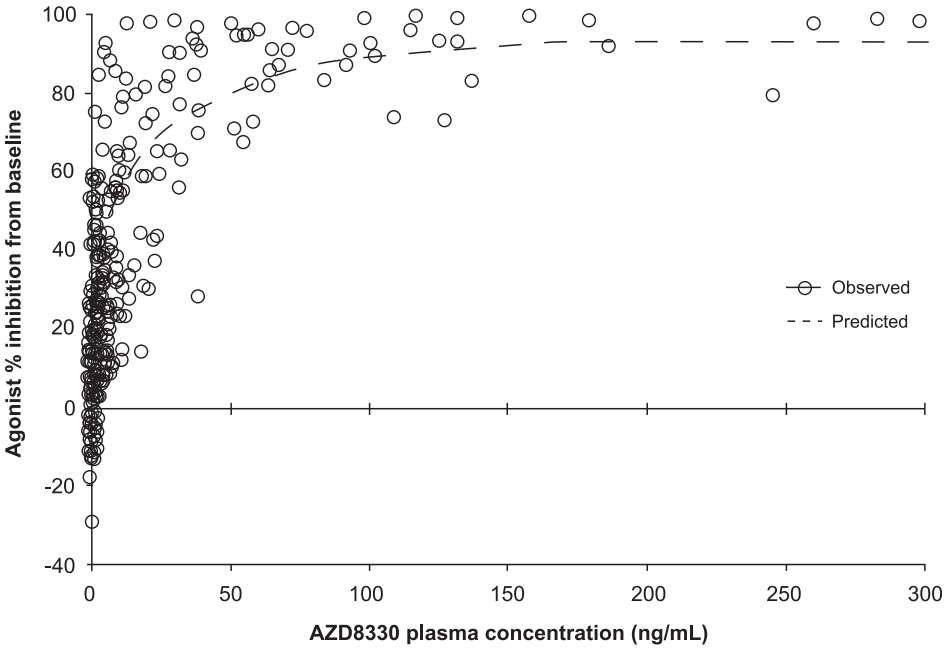

Fig. 2. Pharmacokinetic/pharmacodynamic dose response: E_{max} model.

were not unexpected. One patient in the 12 mg OD cohort experienced an SAE of LV dysfunction. However, no additional cardiac results of concern were observed in the expanded 12 mg OD cohort, confirming this dose as tolerable.

The DLTs of mental status changes in the 40 mg OD cohort were an important and unexpected finding as these have been reported infrequently with selumetinib,[1,9] trametinib,[2,3] RO4987655[5] and CI-1040.[10] Hallucination has been reported occasionally with PD-0325901.[7] It is possible that these observations may reflect blood–brain barrier penetration by AZD8330 or differences in PK characteristics between AZD8330 and other MEK inhibitors. However, the first suggestion is unlikely, as preclinical tests in rats show minimal AZD8330 penetration into the central nervous system (data on file, AstraZeneca, 2007). Any potential link between PK and toxicity was not tested for in this study and cannot be directly compared with other MEK inhibitors, so this remains a matter of speculation.

Preclinical studies demonstrated that AZD8330 OD and BID dosing schedules had similar efficacy in xenograft models.[6] A 20 mg BID dose was evaluated in this study to minimise the mental status change AEs observed in 40 and 60 mg cohorts, whilst maintaining sufficient pERK inhibition to optimise clinical efficacy. However, the results failed to indicate a relationship between mental status change AEs and exposure, as measured by C_{max} and $AUC_{0-\infty}$ on days 1 and 15.

The relatively low toxicity known to be associated with MEK inhibition suggests that the tissue drug concentrations in this study may have been inadequate to achieve sufficient target inhibition, but it is not possible to confirm this directly. PK/PD data confirmed dose-dependent modulation of pERK in PBMCs at doses $\geqslant 3$ mg. Twelve hours after the second dose of the day, pERK was reduced to 66% of baseline at the MTD. This level of inhibition in a surrogate tissue is comparable to that seen with selumetinib, which demonstrated mean pERK inhibition of 51% maintained through BID dosing.[1] However, tumour biopsies, which might have confirmed parallel target tumour inhibition, were not performed and so the actual inhibition of target in the tumour is unknown.

The clinical activity observed with AZD8330 (partial response $n = 1$; stable disease >3 months, $n = 22$; 26.8%), was similar to that reported in a phase I study of selumetinib (stable disease at day 56, $n = 19$; 33.0%).[1] Oral AZD8330 was rapidly absorbed and exposure increased approximately proportionally with dose across the dose range 0.5–60 mg OD. Given the preclinical potency of AZD8330 (7 nM),[6] it was expected that clinical efficacy would be observed at low exposure levels. Consequently, the minimal clinical activity observed in our patient population is striking, considering the high proportion of patients with melanoma and therefore presumed high frequency of *BRAF* mutations. This observation supports the hypothesis that AZD8330 tumour penetration and pathway inhibition may have been insufficient.

Biopsies were not mandated in this study, nor were tests for tumour mutational status. Selumetinib and trametinib have shown clinical responses in *KRAS*-mutant non-small cell lung cancer[4] and *BRAF*-mutant melanoma,[11] respectively, and indeed, one patient with *BRAF*-positive melanoma and another with *BRAF*-positive papillary thyroid cancer had stable disease for 6 and 12 months, respectively in this study.

In conclusion, AZD8330 demonstrated a manageable toxicity profile with fewer class-effect AEs compared with other MEK inhibitors. Target inhibition, as measured by reduction of pERK phosphorylation in PBMCs, was demonstrated with very modest evidence of clinical activity. MEK inhibitors are showing genuine promise in the treatment of advanced malignancies, with associated AEs that require clinical consideration. Data from this study will expand our understanding of the full range of MEK inhibitor toxicities.

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Conflict of interest statement

The Fox Chase Cancer Center (R.B.C.), University of Texas MD Anderson Cancer Center (R.K.) and R.K. received a grant from AstraZeneca. R.K. received funding for consultancy, honoraria and travel from AstraZeneca. M.C., M.L. and I.S. are AstraZeneca employees and hold stocks/shares with the company. D.G. is a contract employee of AstraZeneca. S.A. and M.N. reported no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejca.2013.01.013.

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