Phase I/II multicenter study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of AZD4877 in patients with refractory acute myeloid leukemia

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Summary Eg5 (kinesin spindle protein) is a microtubule motor protein, essential for centrosome separation during mitosis. This Phase I/II, open-label, multicenter, two-part study investigated AZD4877, a potent Eg5 inhibitor, in patients with acute myeloid leukemia. Primary objectives were to determine the maximum tolerated dose (MTD) (part A), assess efficacy (part B) and determine the pharmacokinetic profile (parts A and B). Secondary objectives included assessment of safety and tolerability. AZD4877 was administered at a range of doses (2, 4, 7, 10, 13, 16 and 18 mg/day) as a 1-hour intravenous infusion on three consecutive days of a continuous 2-week schedule. The MTD in part A was defined as 16 mg/day based on dose-limiting stomatitis at 16 and

18 mg/day, hyperbilirubinemia at 16 mg/day and palmar-plantar erythrodysesthesia syndrome at 18 mg/day. Systemic exposure to AZD4877 generally increased with increasing dose whereas half-life was not dose dependent. No evaluable patients experienced a complete remission (CR) or CR with incomplete blood count recovery (CRi), demonstrating no evidence of AZD4877 efficacy in this population. Evidence of monoastars in all but the 4 mg/day dose group provided proof of mechanism for AZD4877. This study was terminated due to lack of efficacy. (ClinicalTrials.gov identifier NCT00486265).

Keywords AML · Eg5 inhibitor · Phase I/II · Safety · MTD · Pharmacokinetics

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Introduction

Acute myeloid leukemia (AML) accounts for 12,330 of the 43,050 new cases of leukemia diagnosed every year in the United States [1]. The primary goal of treatment in patients with AML is to achieve complete remission. This is normally accomplished by initial induction treatment with cytotoxic chemotherapy agents. Although complete remission can be achieved in the majority of young adult patients, ultimate cure depends on disease eradication through the administration of post-remission chemotherapy. Forty to fifty percent of older patients can achieve a complete remission, however less than 10% are long-term survivors, and the cure rate of older patients (which represent the majority of patients with AML) has only minimally improved over the past three decades [2, 3]. Thus, there is a need to develop treatments for AML that are more effective, especially for the elderly who are unable to tolerate intensive chemotherapy regimens.

A rationale has been proposed for targeting the mitotic kinesin Eg5 for treatment of patients with AML; Eg5, an ATP-dependent microtubule motor protein is required for the correct formation of the mitotic spindle; expression of Eg5 is elevated in a number of human proliferative tissues, most notably the bone marrow, as well as in a number of solid tumors and leukemias [4–6]. Indeed, inhibition of Eg5 has been shown to induce the formation of monopolar mitotic spindles ('monoasters') in replicating cells as a result of activation of the spindle assembly checkpoint and induction of cell cycle arrest during the mitotic phase, which leads to cell death by apoptosis [7–9]. Furthermore, Eg5 inhibition has been shown to promote cell death in a number of cancer cell lines and tumor xenograft models [10–14]. In addition, the Eg5 inhibitor ARRY-520 has been shown to cause apoptosis in AML progenitor cells in vitro [15].

Unlike first generation antimitotic agents such as the taxanes and vinca alkaloids, Eg5 inhibitors only target cells that are in mitosis. Therefore they are not expected to have an effect on non-proliferating adult tissue such as the peripheral nervous system [16, 17]. The kinesin spindle protein (KSP) Eg5 has emerged as a novel target that has a selective action on the mitotic pathway that acts only on dividing cells. Consequently, treatment with Eg5 agents is not anticipated to cause the neurotoxic side effects commonly observed with traditional antimitotic agents [4, 14].

AZD4877 is a potent inhibitor of Eg5 that has been shown to disrupt mitotic spindle assembly, leading to mitotic arrest and cell death [18]. Consistent with this mode of action, AZD4877 was shown to induce the formation of monoasters and cell death in tumor xenograft models and in a broad range of both solid and hematologic tumor cell lines [18]. Previously reported investigations of monoasters in peripheral blood mono-

nuclear cells (PBMCs) of patients from the present study have shown that monoasters were detected at all dose levels evaluated (2, 7, 10, 13, and 18 mg/day), consistent with pharmacodynamic activity of AZD4877 in patients with AML [19].

The aim of this Phase I/II study was to determine the maximum tolerated dose (MTD) of AZD4877 and to evaluate the efficacy and pharmacokinetic (PK) profile of AZD4877 in patients with recurrent or refractory AML.

Methods

Patients

Patients aged ≥ 18 years were eligible for inclusion in the study if they had relapsed or refractory AML for which standard therapies were not anticipated to result in durable remission (part A), or if they had AML with a maximum of two prior relapses or failure to achieve remission after at least one attempt at induction treatment (part B). Patients were required to have an Eastern Cooperative Oncology Group Performance Status of 0–3 (part A) or 0–2 (part B). Exclusion criteria included patients with promyelocytic AML according to World Health Organization diagnostic criteria; prior allogeneic transplant requiring immunosuppressive therapy; unresolved acute toxicity of Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 grade >1 from previous anticancer therapy; central nervous system irradiation for meningeal leukemia; a white blood cell count $\geq 10 \times 10^9/l$; impaired liver function (serum bilirubin >1.5 times the upper limit of normal [ULN] unless attributed to underlying disease, or alanine aminotransferase [ALT] or aspartate aminotransferase [AST] >3 times the ULN); impaired renal function (creatinine >2.0 mg/dl or creatinine clearance <50 ml/min, estimated by the Cockcroft-Gault equation or derived from 24-hour urine creatinine); evidence of severe or uncontrolled systemic disease.

All patients provided written, informed consent. The study was approved by the independent ethics committee for each trial center and was conducted in accordance with the Declaration of Helsinki [20], consistent with Good Clinical Practice and the AstraZeneca policy on bioethics.

Study design

This was a Phase I/II, open-label, multicenter, two-part study carried out across five sites in North America and Canada (AstraZeneca study identifier: D2782C00007; ClinicalTrials.gov identifier: NCT00486265).

Part A was a Phase I, open-label, dose-escalation study to determine the MTD of AZD4877 (initial dose 2 mg/day) when administered as an induction course of a 1-hour intravenous (iv) infusion, on three consecutive days, at the beginning of a 2-week schedule for a total of two induction courses. Patients could continue on treatment and receive up to four 2-day consolidation courses at continuous 2-week intervals, provided that there was evidence of complete remission. Initial recruitment to each AZD4877 dose cohort was a minimum of three patients, with a maximum of six evaluable patients in each dose level cohort prior to dose escalation/reduction. Dose escalation only occurred when no patients in a cohort of three evaluable patients or one of six evaluable patients experienced a dose-limiting toxicity (DLT). An evaluable patient in part A was defined as a patient who had completed one 3-day induction course of AZD4877 and had been followed until day 15, or had discontinued from study treatment due to disease progression and was observed until day 10, or a patient who experienced a DLT within the initial 15 days of treatment. A minimum of six evaluable patients were to have been treated at the MTD before part B commenced.

Part B was a phase II, open-label study that assessed the efficacy of AZD4877 administered at the MTD defined in part A. In Part B, a Simon optimal two-stage design [21] was planned based on the rate of complete remission (CR) or CR with incomplete blood count recovery (CRi). Nineteen evaluable patients were to be initially enrolled and followed up for a minimum of 28 days and a maximum of 4 months (for patients with a CR or CRi). If three or fewer of these patients achieved a CR or CRi, the study was to be stopped due to lack of efficacy. If four or more patients achieved a CR or CRi, an additional 14 patients were to be enrolled and treated. In part B, for the purpose of determining efficacy, an evaluable patient was defined as a patient who had received a minimum of one 3-day induction course of AZD4877. For evaluation of safety and tolerability patients must have received at least one dose of AZD4877.

In both parts A and B, patients who achieved CR or CRi after one or two induction courses could remain on the study to receive a maximum of four 2-day consolidation courses administered 7–21 days after recovery of blood cell counts to CTCAE grade ≤ 1 .

The initial recruitment into part B of this open-label study was rapid. Unvalidated reports from the study sites indicated that none of the recruited patients had experienced a CR or CRi. Therefore recruitment into part B of the study was suspended in May 2009. Part B was designed to test the alternative hypothesis that the rate of CR or CRi was 0.35 or above, as a sign of efficacy, versus the null hypothesis that this rate was 0.15 or below. No CR or CRi responses were seen in the first eight patients enrolled. If the alternative hypothesis were true (ie $P[\text{CR} + \text{CRi}] = 0.35$

or above), then the chance of seeing zero CR or CRi responses would have been $(1-0.35)^8 = 0.032$ (3.2%), or less. Also, with 0 out of eight patients responding, only 3.2% of the Bayesian posterior distribution of the $P(\text{CR} + \text{CRi})$ is above 0.35 (using non-informative prior). Therefore, based on the data from eight patients, it was considered unlikely that $P(\text{CR} + \text{CRi})$ is 0.35 or above, and the study was discontinued. Nine evaluable patients were enrolled at study termination.

Objectives

The primary objectives of this study were to identify the MTD of AZD4877 based on assessment of DLT (part A), assess the efficacy of AZD4877 based on the rate of CR and CRi (part B), and determine the PK profile of AZD4877 (parts A and B). The secondary objectives were to evaluate the safety and tolerability of AZD4877 (parts A and B) and to further assess the effect of AZD4877 on the rate and duration of CR, CRi, partial remission (PR) and overall response (part B). Exploratory objectives included the evaluation of the mechanism of action of AZD4877 by assessment of monoaster formation in PBMCs.

Assessments

The MTD of AZD4877 was defined as the highest dose at which no more than one of six evaluable patients experienced a DLT during the first induction course. DLTs were defined as CTCAE grade 3 or 4 hemolysis; CTCAE grade 3 or 4 non-hematological toxicity; AST or ALT CTCAE grade 3 (for >7 days), or grade 4 (of any duration) except for patients with grade 3 transaminases due to leukemic organ involvement at baseline; CTCAE grade 2 diarrhea for >4 days despite aggressive outpatient management; CTCAE grade 3 or 4 vomiting for >24 h despite suitable anti-emetics, or any other toxicity related to study treatment considered to be a DLT by the investigator.

Safety and tolerability were evaluated by assessment of AEs according to CTCAE version 3.0. Samples for laboratory assessments (clinical chemistry and hematology) were taken pre-dose on days 1, 2, 3 (hematology only) and on day 8 of induction courses 1 and 2, and urinalysis parameters were measured pre-dose on day 1 and at the end of the infusion on day 3 of induction courses 1 and 2.

Blood samples were collected for the determination of plasma concentrations of AZD4877 pre-dose on days 1, 2 and 3, and on days 4, 5 and 8 of induction course 1. PK parameters were evaluated following day 3 of the first induction course and included maximum plasma drug concentration (C_{max}), plasma drug concentration 24 h after administration ($C_{24\text{h}}$), area under the concentration–time curve from zero to infinity ($\text{AUC}_{0-\infty}$), area under the

concentration–time curve from zero to 24 h (AUC_{0-24h}) and half-life ($t_{1/2\lambda_z}$) associated with the terminal elimination rate constant (λ_z) of a semi-logarithmic concentration–time curve. Non-compartmental methods were used for the evaluation of the plasma concentration–time data and C_{max} was determined by inspection of the concentration–time profiles. Where possible, λ_z was calculated by log-linear regression of the terminal portion of the concentration–time profiles, and $t_{1/2\lambda_z}$ was defined as $0.6932/\lambda_z$. AUC_{0-24h} was determined using the linear trapezoidal rule and, where appropriate, AUC_{0-24h} was extrapolated to infinity using λ_z to obtain $AUC_{0-\infty}$. The methods for the PK parameter assessments and calculations reported in this study have been described previously [22].

Efficacy of AZD4877 was determined by investigator assessment of bone marrow response using modified Cheson response criteria for AML [23, 24]. Bone marrow response was categorized as CR, CRi, or PR and the duration of response was defined as time from first documentation of CR, or CRi to relapse.

The presence of monoasters in PBMCs was assessed pre-dose, 6–8 and 24 h after the first infusion of AZD4877. PBMCs prepared from whole blood samples using cell preparation tubes (Vacutainer CPTs; Becton Dickinson, Franklin Lakes, NJ, USA) were washed in phosphate buffered saline, centrifuged onto glass slides and air dried. Immunohistochemical staining for α -tubulin was performed on the Ventana Discovery XT system slide stainer using the OmniMap detection kit (Ventana, Tucson, AZ, USA) according to the manufacturer's instructions. Total cell counts were determined by image analysis using Aperio IHC Nuclear Algorithm software (Aperio Technologies Inc. Vista, CA, USA), and monoasters were scored manually. Monoaster counts were expressed as number per 10,000 PBMCs with a mean of 100,000 cells counted for each assessment.

Statistical analysis

Due to early termination of part B, and lack of CR and CRi responses, no formal statistical analyses of efficacy were conducted. Statistical analyses were restricted to summaries of all data from all treated patients to assess safety, tolerability, PK, pharmacodynamics and initial indications of anti-leukemic activity of AZD4877.

Results

Patient characteristics

A total of 47 patients were enrolled in the study, 39 of whom received at least one dose of AZD4877 and were evaluable for safety (30 patients in part A and nine

patients in part B) (Table 1). Two patients in part A were excluded from DLT and PK assessments because they did not receive the complete 3-day induction course of AZD4877, one because of voluntary discontinuation from AZD4877 treatment (2 mg/day dose cohort) and the other because of discontinuation due to CTCAE grade 3 hyperbilirubinemia (16 mg/day dose cohort). All patients recruited into part B were evaluable for safety, efficacy and PK assessment.

The mean age of patients was 62.4 years (range 23–84 years), 32 patients (82%) were Caucasian, and 26 patients (67%) were male. All patients received prior treatment regimens; 18 patients (46%) had initial induction failure, and 21 patients (54%) did not. Thirty-two patients (82%) discontinued the study due to a lack of therapeutic response, three (7.7%) patients voluntarily discontinued, two patients (5.1%) discontinued due to an AE (hyperbilirubinemia; Stevens–Johnson syndrome), one patient (2.6%) died due to disease progression and one patient (2.6%) discontinued for other reasons.

Twenty-two patients (56%) received a single, complete, 3-day induction course (including five patients in part B) and 15 patients (39%) completed two induction courses (including five patients in part B). Two patients proceeded to receive two consolidation courses each, one patient each in the 7 mg/day and 10 mg/day dose cohorts. The median (range) number of doses of AZD4877 received was three (1–10) and the median (range) total dose received was 48 mg (2–108 mg).

Safety profile

During the first induction course, three patients experienced a total of four DLTs, one patient receiving AZD4877 16 mg/day and two patients receiving AZD4877 18 mg/day (Table 2). The MTD for AZD4877 was defined as 16 mg/day.

All patients experienced at least one AE, the most commonly reported were diarrhea ($n=18$), hypokalemia ($n=16$), nausea ($n=14$), fatigue ($n=12$) and hypocalcemia ($n=12$). Twenty-four patients experienced CTCAE grade ≥ 3 , of which hypokalemia ($n=8$) was the most commonly reported (Table 3). Serious adverse events (SAEs) occurred in 13 patients across all dose groups. Mucosal inflammation was the only SAE reported in more than one patient, occurring in three patients in part B. Seven patients had a total of nine SAEs that were considered to be causally related to AZD4877 treatment (by patient, these events were: atrial flutter; diverticulitis and hyperbilirubinemia; mucosal inflammation [two separate patients]; mucosal inflammation and Stevens–Johnson syndrome; palmar–plantar erythrodysesthesia syndrome; and stomatitis). Six patients died due to disease progression (only one of whom discontinued the study due to death), two of whom had fatal SAEs reported as

Table 1 Patient baseline demographics

	AZD4877									
	Part A					Part B				
	2 mg/day (n=6)	4 mg/day (n=3)	7 mg/day (n=4)	10 mg/day (n=3)	13 mg/day (n=3)	16 mg/day (n=7)	18 mg/day (n=4)	16 mg/day (n=9)	Total (n=39)	
Age, years										
Mean (SD)	64.2 (11.5)	67.7 (17.0)	54.8 (27.0)	63.7 (8.5)	67.3 (10.1)	59.0 (16.6)	65.3 (14.3)	62.0 (10.8)	62.4 (14.1)	
Range	47–75	48–78	25–84	54–70	58–78	23–73	44–75	48–79	23–84	
Gender, n (%)										
Male	4 (67)	1 (33)	3 (75)	3 (100)	1 (33)	6 (86)	3 (75)	5 (56)	26 (67)	
Female	2 (33)	2 (67)	1 (25)	0	2 (67)	1 (14)	1 (25)	4 (44)	13 (33)	
Race, n (%)										
Caucasian	4 (67)	1 (33)	3 (75)	2 (67)	3 (100)	7 (100)	4 (100)	8 (89)	32 (82)	
Black African/American	0	1 (33)	0	1 (33)	0	0	0	1 (11)	3 (8)	
Asian	0	1 (33)	1 (25)	0	0	0	0	0	2 (5)	
Other	2 (33)	0	0	0	0	0	0	0	2 (5)	
ECOG performance status, n (%)										
Normal activity	0	1 (33)	1 (25)	1 (33)	0	2 (29)	0	1 (11)	6 (15)	
Restricted activity	3 (50)	1 (33)	2 (50)	2 (67)	2 (67)	5 (71)	3 (75)	7 (78)	25 (64)	
In bed ≤50% of the time	2 (33)	1 (33)	1 (25)	0	0	0	0	1 (11)	5 (13)	
In bed >50% of the time	1 (17)	0	0	0	1 (33)	0	1 (25)	0	3 (8)	
Previous induction attempts, n (%)										
1	0	0	1 (25)	0	1 (33)	2 (29)	1 (25)	3 (33)	8 (21)	
2	0	0	1 (25)	0	1 (33)	3 (43)	0	1 (11)	6 (15)	
3	0	0	1 (25)	0	0	0	1 (25)	1 (11)	3 (8)	
5	1 (17)	0	0	0	0	0	0	0	1 (3)	
Missing	5 (83)	3 (100)	1 (25)	3 (100)	1 (33)	2 (29)	2 (50)	4 (44)	21 (54)	

ECOG, Eastern Cooperative Oncology Group; SD, standard deviation

Table 2 Dose-limiting toxicities

	AZD4877 dosage (mg/day)	Number of patients experiencing DLTs/number of evaluable patients in group	DLTs (CTCAE grade)
Part A	2	0/5	None
	4	0/3	None
	7	0/4	None
	10	0/3	None
	13	0/3	None
	16	1/5	▪ Hyperbilirubinemia (grade 3) and stomatitis (grade 3)
	18	2/4	▪ Palmar–plantar erythrodysesthesia syndrome (grade 3) ▪ Stomatitis (grade 3)
Part B	16	0/9	None

CTCAE, Common Terminology Criteria for Adverse Events version 3.0; DLT, dose-limiting toxicity

secondary causes of death (cerebral hemorrhage and fungal pneumonia in the 2 and 10 mg/day dose cohorts, respectively). One patient discontinued the study due to CTCAE grade 3 hyperbilirubinemia, which was considered by the investigator to be causally related to AZD4877 treatment.

CTCAE grade ≥ 3 clinical chemistry values were reported in 23 patients. The events included high magnesium, high potassium and low serum albumin. These abnormalities showed no apparent dose relationship with AZD4877 and were consistent with those typically found in patients with AML. No clinically significant changes in vital signs or ECG were reported.

Pharmacokinetics, pharmacodynamics and efficacy

Thirty-seven patients had evaluable PK data (Table 4). Exposure to AZD4877 ($C_{\max}$, C_{24h} , $AUC_{0-\infty}$ and AUC_{0-24}) increased in an approximately dose-proportional manner, whereas the $t_{1/2}$ of AZD4877 did not appear to be dose dependent.

No remissions or responses according to the Cheson criteria were reported during parts A or B of the study. In part B, all nine patients had treatment failure, which was attributed to resistant disease. The study was terminated due to a lack of efficacy following the evaluation of the nine patients in part B.

Table 3 Number (%) of patients experiencing CTCAE grade ≥ 3 adverse events reported in ≥ 2 patients overall

Adverse event (AE) n (%)	AZD4877 dose								Total (n=39)
	Part A							Part B	
	2 mg/day (n=6)	4 mg/day (n=3)	7 mg/day (n=4)	10 mg/day (n=3)	13 mg/day (n=3)	16 mg/day (n=7)	18 mg/day (n=4)	16 mg/day (n=9)	
Any CTCAE grade ≥ 3 event	6 (100)	0	2 (50)	2 (67)	1 (33)	5 (71)	4 (100)	4 (44)	24 (62)
Hypokalemia	0	0	0	1 (33)	1 (33)	3 (43)	1 (25)	2 (22)	8 (21)
Hypophosphatemia	0	0	1 (25)	0	0	1 (14)	0	1 (11)	3 (8)
Stomatitis	0	0	0	0	0	1 (14)	2 (50)	0	3 (8)
Fatigue	1 (17)	0	0	1 (33)	0	0	0	0	2 (5)
Mucosal inflammation	0	0	0	0	0	0	0	2 (22)	2 (5)
Dysphagia	0	0	0	1 (33)	1 (33)	0	0	0	2 (5)
Pneumonia	1 (17)	0	1 (25)	0	0	0	0	0	2 (5)
Hyperbilirubinemia	1 (17)	0	0	0	0	1 (14)	0	0	2 (5)

CTCAE, Common Terminology Criteria for Adverse Events version 3.0

Table 4 Summary of AZD4877 pharmacokinetic parameters following day 3 of the first induction course

Parameter, geometric mean (CV%) [number of patients evaluated]	AZD4877 dose								
	Part A							Part B	
	2 mg/day (n=5)	4 mg/day (n=3)	7 mg/day (n=4)	10 mg/day (n=3)	13 mg/day (n=3)	16 mg/day (n=6)	18 mg/day (n=4)	16 mg/day (n=9)	Total (n=37)
C_{max} (ng/ml)	11.3 (9.4) [3]	19.3 (22.1) [2]	41.9 (45.6) [4]	123 (157) [3]	114 (59.2) [3]	99.4 (24.2) [5]	200 (92.2) [4]	87.1 (130) [7]	71.2 (168) [31]
C_{24h} (ng/ml)	2.9 (72.6) [5]	4.5 (85.6) [2]	8.0 (29.6) [4]	12.3 (14.3) [2]	19.0 (74.5) [3]	23.4 (28.2) [5]	48.0 (114) [4]	23.3 (18.1) [5]	13.2 (148) [30]
$AUC_{0-\infty}$ (ng.h/ml)	–	–	750 (53.1) [3]	–	571 [1]	1290 (0.8) [2]	–	–	860 (42.8) [6]
AUC_{0-24} (ng.h/ml)	112 (61.7) [5]	129 (76.0) [2]	439 (60.4) [4]	402 (3.1) [2]	842 (65.2) [3]	729 (21.1) [6]	1520 (48.3) [4]	921 (127) [7]	521 (117) [33]
$t_{1/2\lambda_z}$ * (h)	–	–	16.7 (2.9) [3]	–	18.9 [1]	17.0 (1.4) [2]	–	–	17.1 (2.1) [6]

$AUC_{0-\infty}$, area under the plasma concentration–time curve from zero to infinity; AUC_{0-24} , area under the plasma concentration–time curve from zero to 24 h; C_{24h} , plasma drug concentration 24 h after administration; C_{max} , maximum plasma drug concentration; CV, coefficient of variance; $t_{1/2\lambda_z}$, half-life; –, mean not calculable; *data presented as arithmetic mean (standard deviation)

PBMCs from 12 patients in part A were evaluated for the exploratory endpoint of monoaster detection. Monoasters were detected in nine patients 6 h after the start of AZD4877 treatment on day 1 ($n=1$ patient, 2 mg/day, 7 mg/day, 10 mg/day and 13 mg/day; $n=2$ patients, 18 mg/day; $n=3$ patients, 16 mg/day) and in four patients ($n=2$ patients, 16 mg/day; $n=2$ patients, 18 mg/day) 24 h following AZD4877 treatment on day 2 (Fig. 1).

Discussion

The MTD of AZD4877, when administered as a 1-hour iv infusion on three consecutive days, was defined as 16 mg/day. In a study of patients with solid tumors, DLTs of neutropenia were observed at doses of greater than 30 mg, administered as three weekly 1-hour iv infusions in a 28-day schedule [25]. However, in the current study neutropenia was not reported as a DLT and the DLTs were stomatitis (16 and 18 mg/day cohorts), hyperbilirubinemia (16 mg/day cohort) and pal-

mar–plantar erythrodysesthesia syndrome (18 mg/day cohort). The AEs of stomatitis and mucosal inflammation occurred in a dose-dependent manner and are consistent with the mechanism of action of AZD4877 as this agent directly targets proliferating tissues. All patients had at least one AE, with diarrhea, hypokalemia, nausea, fatigue and hypocalcemia the most commonly reported. Hypokalemia, hypophosphatemia and stomatitis accounted for the highest number of CTCAE grade ≥ 3 events. Mucosal inflammation was the only SAE reported in more than one patient. Other commonly reported AEs (hypokalemia and hypocalcemia) were consistent with those in patients with AML [26].

PK parameters in the present study were consistent with data from another clinical trial of AZD4877 [25]. Although there was some variability across the dose range, systemic exposure to AZD4877 was approximately dose proportional, supporting an approximately linear PK profile. The half-life of AZD4877 was approximately 17 h. The variability in PK parameters observed in the current study may be due to the small number of patients in each dose group.

Fig. 1 Monoaster formation in PBMCs from a representative patient receiving **a.** AZD4877 2 mg/day and **b.** AZD4877 18 mg/day. Footnote to fig: Arrows indicate monoasters; x400 magnification

Proof of mechanism was confirmed by the detection of monoasters in PBMCs at all dosage levels studied. There were no evaluable patients at the AZD4877 4 mg dose, so this cohort was not studied. On day 1, monoasters were detected in PBMCs from patients at all doses in a dose-dependent manner, whereas monoasters were only detected in the 16 and 18 mg/day dose cohorts on day 2, signifying a greater duration of response at these doses. Despite evidence of monoasters, there were no complete or partial remissions to AZD4877 treatment. Limited clinical efficacy has also been reported in another clinical trial of AZD4877 [25], and in trials of other Eg5 agents in patients with solid tumors and malignant melanoma [27–31]. This may be due to insufficiently high and/or sustained exposure to Eg5 agents because of DLTs. Notably, in a Phase I trial of AZD4877 in patients with advanced solid malignancies, the longest duration of stable disease was associated with the highest dose studied [25]. Although AZD4877 and Eg5 inhibitors in general have proven highly effective at killing tumor cells in preclinical models, their efficacy in humans appears to be limited by an insufficient therapeutic window. Ongoing clinical trials of other Eg5 inhibitors such as Array 520 and 4SC-205 will confirm if this is true for all drugs of this type.

In conclusion, AZD4877 was generally well tolerated, and demonstrated an acceptable PK profile in patients with AML. Several KSP inhibitors such as MK0371, Array 520 and SB-715992/ispinesib are currently in clinical trials for various solid tumors. Ispinesib has demonstrated clinical activity in a Phase II study of patients with platinum/taxane refractory or resistant relapsed ovarian cancer [32]. In the current Phase I study of patients with AML, there was evidence of monoaster formation, which was indicative of the proof of mechanism for Eg5 inhibition, however, the lack of clinical efficacy in part B resulted in the early termination of the study. Based on the relative lack of clinical efficacy in this and other completed Phase I and II trials of AZD4877, further development of this agent as an anticancer therapy is not planned. Future challenges of developing KSP inhibitors in leukemia have to carefully consider the kinetics of the disease and include combined synergistic strategies with existing chemotherapeutic agents.

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References

- Jemal A, Siegel R, Xu J, Ward E (2010) Cancer statistics, 2010. *CA Cancer J Clin* 60:277–300
- Kantarjian H, O'Brien S, Cortes J, Giles F, Faderl S, Jabbour E, Garcia-Manero G, Wierda W, Pierce S, Shan J, Estey E (2006) Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome. *Cancer* 106:1090–1098
- Kantarjian H, Ravandi F, O'Brien S, Cortes J, Faderl S, Garcia-Manero G, Jabbour E, Wierda W, Kadia T, Pierce S, Shan J, Keating M, Freireich EJ (2010) Intensive chemotherapy does not benefit most older patients (age 70 years or older) with acute myeloid leukemia. *Blood* 116:4422–4429
- Sakowicz R, Finer JT, Beraud C, Crompton A, Lewis E, Fritsch A, Lee Y, Mak J, Moody R, Turincio R, Chabala JC, Gonzales P, Roth S, Weitman S, Wood KW (2004) Antitumor activity of a kinesin inhibitor. *Cancer Res* 64:3276–3280
- Hedge PS, Cogswell J, Carrick J, Jackson J, Wood KW, Eng WK, Brawner M, Huang PS, Bergsma D (2003) Differential gene expression analysis of kinesin spindle protein in human solid tumors. *Proceedings of the American Society of Clinical Oncology* 22:abst 535
- Castillo A, Morse HC III, Godfrey VL, Naeem R, Justice MJ (2007) Overexpression of Eg5 causes genomic instability and tumor formation in mice. *Cancer Res* 67:10138–10147
- Blangy A, Lane HA, d'Herin P, Harper M, Kress M, Nigg EA (1995) Phosphorylation by p34cdc2 regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation in vivo. *Cell* 83:1159–1169
- Masuda A, Maeno K, Nakagawa T, Saito H, Takahashi T (2003) Association between mitotic spindle checkpoint impairment and susceptibility to the induction of apoptosis by anti-microtubule agents in human lung cancers. *Am J Pathol* 163:1109–1116
- Taylor SS, McKeon F (1997) Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. *Cell* 89:727–735
- Hayashi N, Koller E, Fazli L, Gleave ME (2008) Effects of Eg5 knockdown on human prostate cancer xenograft growth and chemosensitivity. *Prostate* 68:1283–1295
- Kapoor TM, Mayer TU, Coughlin ML, Mitchison TJ (2000) Probing spindle assembly mechanisms with monastrol, a small molecule inhibitor of the mitotic kinesin, Eg5. *J Cell Biol* 150:975–988
- Liu M, Yu H, Huo L, Liu J, Li M, Zhou J (2008) Validating the mitotic kinesin Eg5 as a therapeutic target in pancreatic cancer cells and tumor xenografts using a specific inhibitor. *Biochem Pharmacol* 76:169–178
- Nakai R, Iida S, Takahashi T, Tsujita T, Okamoto S, Takada C, Akasaka K, Ichikawa S, Ishida H, Kusaka H, Akinaga S, Murakata C, Honda S, Nitta M, Saya H, Yamashita Y (2009) K858, a novel inhibitor of mitotic kinesin Eg5 and antitumor agent, induces cell death in cancer cells. *Cancer Res* 69:3901–3909
- Zhang Y, Xu W (2008) Progress on kinesin spindle protein inhibitors as anti-cancer agents. *Anticancer Agents Med Chem* 8:698–704
- Carter BZ, Mak DH, Wassner R, Gross S, Schober WD, Estrov Z, Kantarjian H, Andreev E (2009) Inhibition of KSP by ARRY-520 induces cell cycle block and cell death via the mitochondrial pathway in AML cells. *Leukemia* 23:1755–1762
- Orr GA, Verdier-Pinard P, McDaid H, Horwitz SB (2003) Mechanisms of taxol resistance related to microtubules. *Oncogene* 22:7280–7295
- Mayer TU, Kapoor TM, Haggarty SJ, King RW, Schreiber SL, Mitchison TJ (1999) Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen. *Science* 286:971–974
- Pinzon-Ortiz MC, Cao A, Sheehy A, Pablo L, McEachern K, Hylander-Gans L, Wu K, Reimer C, Morosini D, McCoon P, Huszar D (2010) Characterization of the kinesin spindle protein inhibitor AZD4877. *Proc Am Assoc Cancer Res* 51:abst 4429

19. Borthakur G, Faderl S, Ravandi F, Padmanabhan S, Stock W, Wu K, Li J, Curt G, Tallman M, Minden M (2009) Clinical, pharmacokinetic (PK), and pharmacodynamic findings from a phase I trial of an Eg5 inhibitor (AZD4877) in patients with refractory acute myeloid leukemia (AML). *J Clin Oncol* 27(15S):abst 3580
20. World Medical Association (WMA). Declaration of Helsinki. Ethical principles for medical research involving human subjects. Adopted by the 18th WMA General Assembly, Helsinki, Finland, June. 1964. Available at: <http://www.wma.net/en/30publications/10policies/b3/index.html>
21. Simon R (1989) Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 10:1–10
22. Rowland M, Tozer TN (1995) Clinical pharmacokinetics: concepts and applications, 3rd edn. Williams & Wilkins, Lippincott
23. Cheson BD, Bennett JM, Kopeccky KJ, Buchner T, Willman CL, Estey EH, Schiffer CA, Doehner H, Tallman MS, Lister TA, Lo-Coco F, Willemze R, Biondi A, Hiddemann W, Larson RA, Lowenberg B, Sanz MA, Head DR, Ohno R, Bloomfield CD (2003) Revised recommendations of the international working group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. *J Clin Oncol* 21:4642–4649
24. Kantarjian HM, Gandhi V, Kozuch P, Faderl S, Giles F, Cortes J, O'Brien S, Ibrahim N, Khuri F, Du M, Rios MB, Jeha S, McLaughlin P, Plunkett W, Keating M (2003) Phase I clinical and pharmacology study of clofarabine in patients with solid and hematologic cancers. *J Clin Oncol* 21:1167–1173
25. Infante JR, Sprattlin JL, Kurzrock R, Eckhardt SG, Burris HA, Pulchalski TA, Li J, Wu K, Ochs J, Herbst RS (2008) Clinical, pharmacokinetic (PK), pharmacodynamic findings in a phase I trial of weekly (wkly) intravenous AZD4877 in patients with refractory solid tumors. *J Clin Oncol* 26(15S):abst 2501
26. Milionis HJ, Bourantas CL, Siamopoulos KC, Elisaf MS (1999) Acid-base and electrolyte abnormalities in patients with acute leukemia. *Am J Hematol* 62:201–207
27. Tang PA, Siu LL, Chen EX, Hotte SJ, Chia S, Schwarz JK, Pond GR, Johnson C, Colevas AD, Synold TW, Vasist LS (2008) Phase II study of ispinesib in recurrent or metastatic squamous cell carcinoma of the head and neck. *Invest New Drugs* 26:257–264
28. Knox JJ, Gill S, Synold T, Biagi JJ, Major P, Feld R, Cripps C, Wainman N, Eisenhauer E, Seymour L (2008) A phase II and pharmacokinetic study of SB-715992, in patients with metastatic hepatocellular carcinoma: a study of the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG IND.168). *Invest New Drugs* 26:265–272
29. Lee CW, Belanger K, Rao SC, Petrella TM, Tozer RG, Wood L, Savage KJ, Eisenhauer EA, Synold TW, Wainman N (2008) A phase II study of ispinesib (SB-715992) in patients with metastatic or recurrent malignant melanoma: a National Cancer Institute of Canada Clinical Trials Group trial. *Invest New Drugs* 26:249–255
30. Lee RT, Beekman KE, Hussain M, Davis NB, Clark JI, Thomas SP, Nichols KF, Stadler WM (2008) A University of Chicago consortium phase II trial of SB-715992 in advanced renal cell cancer. *Clin Genitourin Cancer* 6:21–24
31. Beer TM, Goldman B, Synold TW, Ryan CW, Vasist LS, Van Veldhuizen P Jr, Dakhil SR, Lara PN Jr, Drelichman A, Hussain MH, Crawford ED (2008) Southwest Oncology Group phase II study of ispinesib in androgen-independent prostate cancer previously treated with taxanes. *Clin Genitourin Cancer* 6:103–109
32. Shahin MS, Braly P, Rose P, Malpass T, Bailey H, Alvarez RD, Hodge J, Bowen C, Buller R (2007) A Phase II, open-label study of ispinesib (SB-715992) in patients with platinum/taxane refractory or resistant relapsed ovarian cancer. *J Clin Oncol* 25(18S):abst 5562