Targeted therapy in oncology: mechanisms and toxicity

Steeghs, N.

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A phase I pharmacokinetic and pharmacodynamic study of the aurora kinase inhibitor danusertib (PHA-739358) in patients with advanced or metastatic solid tumors

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Abstract

Purpose

Danusertib (PHA-739358) is a small-molecule pan-aurora kinase inhibitor. This phase I dose escalation study was conducted to evaluate safety and tolerability of danusertib with additional pharmacokinetics, biomarker and efficacy assessments.

Patients and methods

Patients with solid tumors refractory to standard therapies or with no standard therapy available were enrolled. Danusertib was administered intravenously on days 1,8,15 every 28 days in 6-hour or 3-hour infusion schedules (6h-ivS, 3h-ivS). Dose levels from 45 mg/m^2 in the 6h-ivS, and from 250 mg/m^2 in the 3h-ivS were studied.

Results

Fifty patients were treated. For the 6h-ivS, the most frequently reported side effects were neutropenia (55%), nausea (25%), anorexia (23%), fatigue (20%), and diarrhea (18%). In the 3h-ivS, fatigue (70%), neutropenia (60%), diarrhea (50%), and nausea (30%) were seen. Non-hematological toxicity was mild to moderate. Neutropenia was dose limiting. The maximum tolerated dose was 330 mg/m^2 for the 6h-ivS and not identified for the 3h-ivS. The systemic exposure to danusertib increased linear with dose. The infusion rate did not appear to influence remarkably the pharmacokinetics of danusertib. Biomarker analysis showed inhibition of histone H3 phosphorylation, indicative of Aurora B inhibition, at doses ≥190 mg/m^2. Stable disease was observed in 23.7% of evaluable patients with disease stabilization ≥6 months in 5 patients.

Conclusions

Dose limiting toxicity of danusertib is neutropenia which was short lasting and generally uncomplicated, with limited non-hematological toxicity. The recommended dose of danusertib for phase II studies is 330 mg/m^2 infused over 6 hours on days 1, 8, 15 every 28 days.

Introduction

Aurora kinases are serine/threonine kinases with a key role in mitosis. The aurora kinase family consists of 3 members, aurora-A, B, and C. Aurora-A is localized to the centrosomes of interphase cells and to the mitotic spindle of cells from prophase throughout telophase, and is required for proper spindle maturation and assembly.[1-3] Aurora-B is critical for chromosomal condensation, the attachment of the microtubules to the kinetochore of chromosomes and for proper execution of cytokinesis.[4,5] Aurora-C is found in the testes where it has a role in spermatogenesis. In addition aurora-C might act as a chromosomal passenger protein that can complement aurora-B kinase function in mitotic cells.[6,7]

Since aurora kinases are largely involved in cell cycle progression and mitosis, which is disturbed in cancer cells, their inhibition is considered to have potential as anti cancer treatment. In vitro, inhibition of aurora-A or aurora-B activity in tumor cells results in impaired chromosome alignment, weakening of the mitotic checkpoint, polyploidy, and subsequent cell death.[8,9]

Danusertib is a potent small-molecule inhibitor of the ATP site of the aurora-A (IC50: 13 nM), aurora-B (IC50: 79 nM) and aurora-C (IC50: 61 nM) serine/threonine kinases.[10,11] The chemical structure of danusertib is demonstrated in Figure 1. Danusertib is active in a wide range of cancer cell lines and xenografts models.[10] In mice, danusertib inhibits phosphorylation of histone H3, a protein implicated in chromosome condensation that is phosphorylated by aurora-B. This effect is observed in skin, bone marrow and xenograft tumors.[12] Therefore, inhibition of histone H3 phosphorylation has been identified as marker of danusertib biological activity. Preclinical pharmacokinetics of danusertib were dose-proportional and time-independent. The major route of metabolism involved the formation of the N-oxide derivative. The N-oxide metabolite was deter-

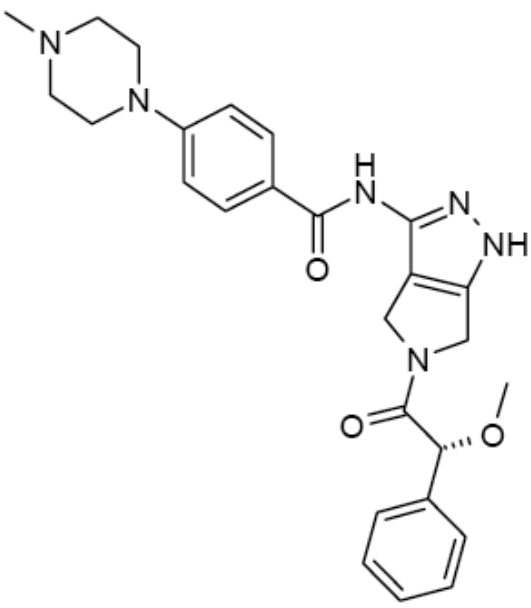


Fig. 1. Chemical structure of PHA-739358

mined to have less than 1% of the activity of the parent compound. Danusertib did not inhibit any cytochrome P450 isoenzymes and was not a potent inhibitor of P-glycoprotein.[11] We performed a phase I pharmacological and biomarker study of danusertib in patients with solid tumors. Objectives of this study were to (1) determine the maximum tolerated dose (MTD) and define dose-limiting toxicities (DLT), (2) characterize safety, (3) characterize pharmacokinetics, (4) analyze biomarkers of biological activity, including histone H3 phosphorylation in skin biopsies, and (5) evaluate preliminary antitumor activity.

Patients and Methods

Eligibility Criteria

Patients with histologically or cytologically confirmed advanced or metastatic solid tumors for whom no standard therapy was available, with an Eastern Cooperative Oncology Group (ECOG) performance status ≤1 were eligible. Other inclusion criteria were: evaluable or measurable disease by RECIST[13]; age ≥18 years; life expectancy ≥12 weeks; tumor progression prior to study entry, adequate bone marrow, liver, and renal function (hemoglobin ≥10.0 g/dl; absolute neutrophil count ≥1,500/mm^3; platelet count ≥100,000/mm^3; total bilirubin ≤1.5x the upper limit of normal (ULN); ALT and AST ≤2.5x ULN, (<5x ULN in case of liver metastases); serum albumin ≥3.0 g/dL; serum creatinine ≤1.5 mg/dL); blood pressure ≤140/90 mm Hg. Exclusion criteria were: previous high-dose chemotherapy requiring bone marrow rescue; known brain or leptomeningeal disease; pregnancy or breast-feeding; active inflammatory bowel disease, bowel obstruction or chronic diarrhea; abnormal left ventricular ejection fraction, thromboembolic events in the year prior to enrollment; ongoing cardiac dysrhythmias grade ≥2; known active infections; any condition that could endanger the safety of the patient.

Written informed consent was obtained from all patients before any study related procedure was performed, and approval from the institutional medical ethical review boards was obtained.

Drug Administration and Dose Escalation Procedure

Danusertib was administered intravenously for 3 consecutive weeks in 4-week cycles. Patients were divided into cohorts with escalating doses, starting with 6h-ivS. After MTD definition with the 6h-ivS, in the attempt of shortening the in hospital-time, two additional cohorts of patients were included to study the 3h-ivS. Based on animal

toxicology and pharmacokinetic data, the starting dose for the 6h-ivS of danusertib was 45 mg/m^2 (target exposure 1/10th of the AUC at MTD in dogs, most sensitive species in toxicology studies). The starting dose for the 3h-ivS was 250 mg/m^2 based on toxicity and pharmacokinetic results of the 6h-ivS. Dosing schedules were based on preclinical animal toxicity studies, with higher doses and/or shorter infusion times resulting in increased bone marrow, gastrointestinal, cardiovascular and renal toxicity.

A two-stage accelerated titration design was adopted. During the initial phase a rapid dose escalation scheme was used with 100% dose increments until occurrence of drug-related first cycle DLT in 1 patient or grade ≥2 drug-related toxicity in ≥2 patients during any treatment cycle. For subsequent dose escalation steps a modified Fibonacci scheme was foreseen with 50, 40 and 33% dose increments in subsequent dose levels.

DLT was defined as grade 4 neutropenia ≥7 days, febrile neutropenia, neutropenic infection, grade 4 thrombocytopenia, grade 3 thrombocytopenic bleeding, and any drug-related grade 3 or 4 non-hematological toxicity (excluding nausea, vomiting or diarrhea not refractory to adequate treatment), decrease in LVEF to ≤40% or a decrease of ≥20% compared to baseline, interruption of infusion due to a diastolic blood pressure increase of >20 mm Hg or to >150/100 mm Hg during drug administration, next cycle delayed by ≥2 weeks, and omission of day 8 and/or 15 dose due to danusertib-related toxicity (after the 250 mg/m^2 6-hour cohort protocol amendment allowed dosing on day 8 and/or 15 in the event of grade 3 uncomplicated neutropenia). If DLT was observed in one patient, three additional patients were recruited at that dose level, with dose escalation proceeding if <2 of 6 patients exhibited DLT. If DLT was observed in ≥2 of 3 or ≥2 of 6 patients, the MTD had been exceeded, and additional patients were recruited at the previous lower dose level.

The MTD was defined as the highest dose level that could be given to 6 patients with no more than 1 patient experiencing DLT. If a patient experienced a drug related DLT, further danusertib administration was withheld in that cycle. If the toxicity resolved to ≤grade 1, the dose was reduced to the previous lower dose level. Otherwise, the patient was withdrawn from the study.

Therapy continued until disease progression or unacceptable toxicity

Pre-treatment Evaluation and Safety Assessment

Pretreatment evaluation consisted of a complete medical history, physical examination, ECOG performance status assessment, vital signs, ECG, blood sample for complete blood count (CBC; hemoglobin, white blood cell count with differential, platelet count) and biochemistry analysis (BUN or blood urea, creatinine, albumin, aspartate aminotransfer-

ase, alanine amintotransferase, bilirubin, alkaline phosphatase, lactate dehydrogenase, sodium, potassium), sample for urinalysis, serum pregnancy test, multigated acquisition (MUGA) scan, chest X-ray and baseline tumor measurements.

On days 1, 8, 15 and 22 of each cycle evaluation consisted of a brief history and physical examination, vital signs, blood samples for CBC and biochemistry, urinalysis, ECG. MUGA scans were repeated after cycle 1, and every even cycle. Response evaluation was performed every 2 cycles according to RECIST[13]. Patients were evaluated weekly for adverse events and toxicity according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 3.0.

Pharmacokinetic Evaluation

Pharmacokinetic (PK) evaluation was performed by collecting blood samples via an indwelling intravenous catheter in the opposite arm of the infusion. In cycle 1, on days 1 and 15 a 5 mL sample was collected pre-dose and at 0.5, 1, 3 and 6 (5 min before end infusion) h after start of the infusion, and 5, 15 and 30 min, 1, 2, 4 and 6 h after the end of the infusion. On days 2-4 and 15-18 blood samples were taken corresponding to 24, 48 and 72 h after the start of infusion. On day 8, blood samples were taken predose and 5 min before the end of infusion. On day 22, one blood sample was taken. In subsequent cycles an abbreviated sampling schedule was used. Urine samples were collected predose and up to 72 h after the first dose of cycle 1.

Pharmacokinetic evaluation was carried out using a non-compartmental approach with the aid of WinNonlin software (version 3.1, Pharsight Inc., Mountain View, CA, USA). Plasma and urine concentrations of danusertib and of its N-oxide metabolite were measured by validated liquid chromatography-tandem mass spectrometry techniques. Detailed methods are described in the online only appendix.

Biomarker Analysis

Skin biopsies for biomarker analysis were performed on day 1 of the first cycle, before start and 10 minutes before end of the infusion. Biopsies were processed for immunohistochemistry (IHC), using an anti-phospho histone H3 antibody, as a measure of aurora-B inhibition.[10,14,15] Detailed methods are described in the online only appendix.
Blood samples for blood pressure biomarker analysis (norepinephrine, epinephrine, endothelin A and B, vascular endothelial growth factor, and angiotensin II) were scheduled to be taken pre-dose and every hour during infusion in cycle 1 and in case of a hypertensive event.

Table 1. Baseline demographics and patient characteristics.

Baseline characteristics	PHA-739358 6-h Infusion N=40	PHA-739358 3-h Infusion N= 10
Gender, n (%)		
Male	29 (73)	8 (80)
Female	11 (28)	2 (20)
Age, years		
Median (range)	54 (22-75)	61 (46-74)
ECOG performance scale, n (%)		
0	6 (15)	3 (30)
1	34 (85)	7 (70)
Previous lines of systemic therapies		
Median (range)	4 (0*-12)	3 (1-6)
Tumor type, n (%)		
Colorectal cancer	13	6
Sarcoma	6	1
Esophageal cancer	4	–
Pancreatic cancer	3	–
Cholangiocarcinoma	2	–
Ovarian cancer	2	–
Prostate cancer	2	–
Renal cancer	2	–
Other		
ACUP	1	1
Adrenal cancer	1	–
Bladder cancer	1	1
Breast cancer	1	–
Mesothelioma	–	1
NSCLC	1	–
Thyroid cancer	1	–

ECOG: Eastern Cooperative Oncology Group,
ACUP: Adenocarcinoma of unknown primary, NSCLC: non small cell lung cancer
* 3 pancreatic cancer, 1 cholangiocarcinoma had only previous surgery

Results

Between June 2004 and September 2007, 52 patients were enrolled. Two patients never started treatment because of clinical deterioration due to rapid tumor progression. Patient characteristics are summarized in Table 1.

The percentage of evaluable patients was 94% for PK analyses, 60% for Histone H3 analyses, 100% for toxicity, and 78% for efficacy. A total of 148 cycles were administered. The median number of cycles per patient was 2 (range 1-28). Dose reductions were required in 12% of patients. Reasons for study discontinuation were lack of efficacy (69%) and adverse events (20%). Two patients withdrew consent, and one patient is still on treatment.

Safety and Tolerability

Dose levels for the 6h-ivS were 45 mg/m^2 (n=3), 90 mg/m^2 (n=7), 135 mg/m^2 (n=4), 190 mg/m^2 (n=4), 250 mg/m^2 (n=10), 330 mg/m^2 (n=8), and 400 mg/m^2 (n=4), and 250 mg/m^2 (n=3), and 330 mg/m^2 (n=7) for the 3h-ivS.

In the 6h-ivS, DLT consisted of grade 2 hypertension leading to interruption of infusion in one patient (90 mg/m^2); febrile neutropenia and grade 3 fatigue in one patient (330 mg/m^2); dose omission due to grade 4 neutropenia in 2 patients (400 mg/m^2). Using the 3h-ivS DLT consisted of dose omissions due to grade 4 neutropenia and grade 3 fatigue (330 mg/m^2).

All treatment-related hematological and non-hematological adverse events are summarized in Table 2.

For the 6h-ivS (total of 120 cycles), most frequently observed drug-related side effects were neutropenia, nausea, anorexia, and fatigue. For the 3h-ivS (total of 28 cycles), most frequently observed drug-related side effects were fatigue, neutropenia, diarrhea, and nausea. Grade 3-4 drug-related events were neutropenia, febrile neutropenia, leucopenia, and fatigue reported at doses of 250 mg/m^2 and higher for the 6h-ivS and fatigue, diarrhea, neutropenia, leucopenia, and dehydration at 330 mg/m^2 of the 3h-ivS. Injection site reactions were reported in 3 patients each with both infusion schedules.

For the 6h-ivS drug-related adverse events requiring dose reduction or omission were mainly due to hematological toxicity and started at 250 mg/m^2 (5 cases). In the 3h-ivS dose reduction was pursued in 1 patient for hematological toxicity (330 mg/m^2). Permanent treatment discontinuation for drug-related toxicity was required in 3 patients, for grade 2 anemia associated with fatigue, pain and nausea (190 mg/m^2, 6h-ivS), grade 1 hypertension (330 mg/m^2, 6h-ivS), and grade 3 fatigue (330 mg/m^2, 3h-ivS).

Neutropenia was uncomplicated except for one case of febrile neutropenia (330 mg/m^2, 6h-ivS). Median time to neutropenia nadir was 15 days and median time to recovery 7 days.

The MTD was 330 mg/m^2 for the 6h-ivS, and not defined for the 3h-ivS. The 250 mg/m^2 dose level was not further expanded to confirm it as the MTD for the 3h-ivS, as available data supported the feasibility of a safe administration of 330 mg/m^2 using the 6h-ivS.

Table 2. Number of patients with treatment-emergent hematological adverse events and/or treatment-related non-hematological adverse events per patient during all cycles. Every * represents 1 patient with dose limiting toxicity (DLT). 2A: 6-hour infusion schedule. 2B 3-hour infusion schedule.

2A:

Adverse Event	Cohort 1 6-h Infusion 45 mg/m² n=3		Cohort 2 6-h Infusion 90 mg/m² n= 7		Cohort 3 6-h Infusion 135 mg/m² n=4		Cohort 4 6-h Infusion 190 mg/m² n=4		Cohort 5 6-h Infusion 250 mg/m² n=10		Cohort 6 6-h Infusion 330 mg/m² n=8		Cohort 7 6-h Infusion 400 mg/m² n=4		Total incidence 6-h Infusion n=40
	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	Any grade n (%)
	Treatment-Emergent Hematological Adverse Events														
Anemia	1	–	4	1	1	–	2	–	6	–	2	1	4	–	22 (55.0)
Leukopenia	1	–	3	–	1	–	3	–	6	3	5	1	1	3	27 (67.5)
Neutropenia	1	–	2	–	1	–	3	–	3	3	2	4	1	2**	22 (55.0)
Febrile neutropenia	–	–	–	–	–	–	–	–	–	–	–	1*	–	–	1 (2.5)
Thrombopenia	2	–	2	–	–	–	1	–	1	2	–	–	1	–	9 (22.5)
	Treatment–Related Non–Hematological Adverse Events														
Any event	2	–	4	–	1	–	3	–	4	3	5	1	1	3	27 (67.5)
GI toxicity															
Anorexia	–	–	1	–	–	–	2	–	2	–	2	–	2	–	9 (22.5)
Constipation	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1 (2.5)
Dehydration	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0 (0.0)
Diarrhea	1	–	1	–	–	–	–	–	1	–	2	–	2	–	7 (17.5)
Dyspepsia	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1 (2.5)
Nausea	–	–	1	–	1	–	–	–	4	–	2	–	2	–	10 (25.0)
Vomiting	–	–	–	–	–	–	–	–	2	–	1	–	–	–	3 (7.5)
Constitutional toxicity															
Fatigue	–	–	2	–	–	–	–	–	3	–	2	1*	–	–	8 (20.0)
Miscelaneous															
Abdominal pain	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1 (2.5)
Alopecia	–	–	–	–	–	–	–	–	1	–	1	–	–	–	2 (5.0)
Dizziness	–	–	–	–	–	–	–	–	1	–	1	–	–	–	2 (5.0)
Headache	–	–	1	–	–	–	–	–	–	–	2	–	–	–	3 (7.5)
Hypertension	–	–	2*	–	–	–	–	–	–	–	1	–	–	–	3 (7.5)
Influenza like	–	–	1	–	–	–	–	–	1	–	–	–	–	–	2 (5.0)
Myalgia	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0 (0.0)
Phlebitis	–	–	–	–	–	–	–	–	–	–	1	–	1	–	2 (5.0)
Somnolence	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0 (0.0)

GI: gastro intestinal

2B:

Adverse Event	Cohort 8 3-h Infusion 250 mg/m^2 n=3		Cohort 9 3-h Infusion 330 mg/m^2 n=7		Total incidence 3-h Infusion n=10
	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Any grade n (%)
	Treatment-Emergent Hematological Adverse Events				
Anemia	2	–	4	–	6 (60.0)
Leukopenia	2	–	2	2	6 (60.0)
Neutropenia	1	–	1	4*	6 (60.0)
Febrile neutropenia	–	–	–	–	0 (0.0)
Thrombopenia	–	–	1	–	1 (10.0)
	Treatment–Related Non–Hematological Adverse Events				
Any event	3	–	2	4	9 (90.0)
GI toxicity					
Anorexia	–	–	2	–	2 (20.0)
Constipation	–	–	2	–	2 (20.0)
Dehydration	–	–	–	1	1 (10.0)
Diarrhea	2	–	1	2	5 (50.0)
Dyspepsia	1	–	1	–	2 (20.0)
Nausea	1	–	2	–	3 (30.0)
Vomiting	1	–	1	–	2 (20.0)
Constitutional toxicity					
Fatigue	2	–	3	2*	7 (70.0)
Miscelaneous					
Abdominal pain	–	–	2	–	2 (20.0)
Alopecia	–	–	1	–	1 (10.0)
Dizziness	–	–	–	–	0 (0.0)
Headache	–	–	1	–	1 (10.0)
Hypertension	–	–	–	–	0 (0.0)
Influenza like	–	–	–	–	0 (0.0)
Myalgia	–	–	2	–	2 (20.0)
Phlebitis	–	–	2	–	2 (20.0)
Somnolence	–	–	2	–	2 (20.0)

Pharmacokinetics

Danusertib pharmacokinetic parameters are summarized in Table 3. Day 1 danusertib plasma concentrations after 6-hour infusion dose of danusertib of a representative patient at each dose level are plot in Figure 2. The pharmacokinetics of danusertib were characterized by high volume of distribution and low to moderate plasma clearance

Table 3. Plasma pharmacokinetic parameters (mean ± SD) of PHA-739358 during cycle 1 (6h and 3h infusion schedules). Percentage coefficient of variation (%CV) and range for the recommended phase 2 dose (RP2D, 330 mg/m^2) in italics.

6-hour infusion schedule						
Day 1						
Dose mg/m^2	Cmax μM	t1/2,z hour	AUC0-∞ μM·hour	CL L/hour	Vz L	CLR L/hour
45 (n=3)	0.83±0.3	17.6±0.8	5.9±2.2	33.4±11.0	857±312	4.46±2.21
90 (n=7)	2.25±0.6	27.2±22	14.0±3.0	27.4±6.8	1010±725	3.50±1.56
135 (n=4)	2.56±1.4	19.5±3.9	13.9±3.6	38.3±10.5	1041±198	5.73±2.42
190 (n=4)	3.86±1.1	24.4±7.8	27.5±6.2	30.0±7.6	1085±565	3.06±1.1
250 (n=10)	4.75±1.6	25.1±13	30.8±9.2	35.1±11.8	1272±645	5.37±2.64
330 (n=7)	5.62±2.5	33.3±17	38.5±11	38.3±12.5	1832±933	5.66±1.84 (n=5)
RP2D %CV	*44.9*	*50.8*	*28.6*	*32.6*	*50.9*	*32.6*
RP2D range	*3.3 - 10.3*	*16.0 - 69.0*	*20.7 - 53.0*	*27.6 - 59.0*	*698 - 3095*	*3.7 - 8.4*
400 (n=4)	6.31±2.3	37.7±22	49.3±11	35.5±9.8	1872±1030	4.65±2.43
45-400 (n=39)	4.00±2.3	27.0±15.4	27.1±15.4	34.0±10.4	1312±752	4.69±2.20
Day 15-Day 1 ratio	0.98		0.94			
3-hour infusion schedule						
Day 1						
Dose mg/m^2	Cmax μM	t1/2,z hour	AUC0-∞ μM·hour	CL L/hour	Vz L	CLR L/hour
250 (n=2)	7.10±0.9	32.3±1.5	28.7±2.4	38.7±10.4	1787±400	3.18±0.59
330 (n=6)	10.10±1.7	28.5±9.8	52.7±30	32.8±14.8	1386±762	1.48±0.53 (n=2)
250-330 (n=8)	9.34±2.0	29.4±8.5	46.7±28	34.3±13.4	1487±687	2.33±1.09
Day 15-Day 1 ratio	0.95		0.75			

Cmax: maximal concentration; t1/2,z: terminal half-life; AUC0-∞ : areas under the curve up to infinite time, CL: systemic clearance, Vz: volume of distribution, CLR: renal clearance, %CV: percentage coefficient of variation.

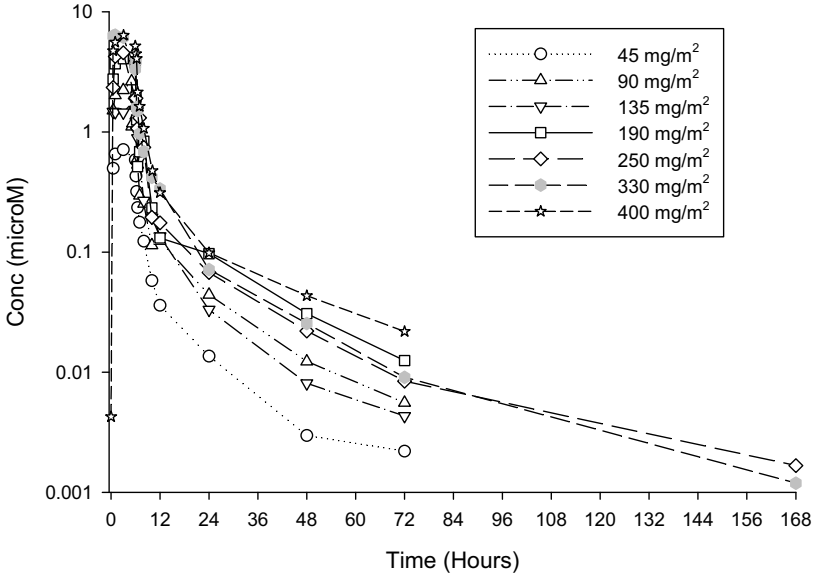


Fig. 2 Representative day 1 individual plasma concentrations (μM) of PHA-739358 after 6 hour infusion of PHA-739358 at each dose level

(range 10-59 L/hour). The half-life was about 30 hours. Accumulation was negligible. Renal clearance accounted for a small proportion of plasma clearance. The metabolite to parent AUC ratio was similar across doses and approximately equal to 1. Metabolite concentrations declined in parallel with those of the parent compound. The systemic exposure to danusertib increased linear with dose (Figure 3A). Pharmacokinetics of danusertib were not influenced by infusion rates (p-values >0.1; independent samples Student's t-test). However, patient numbers were limited. PK data on days 1 and 15 were comparable (p-values >0.1; paired samples t-test; Figure 3B).

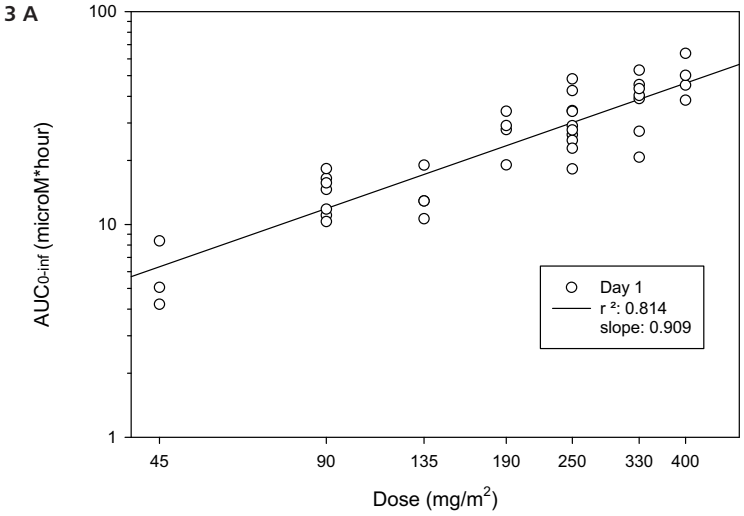
Correlation between Toxicity and Exposure

Figure 4 shows a positive correlation between the percentage decrease in neutrophil counts in cycle 1 in function of the AUC, thus demonstrating that a higher AUC is related to a greater decrease in neutrophil counts during danusertib treatment.[16]

Biomarker Analysis

Histone H3 phosphorylation in skin

Pre-and on-treatment skin biopsies were obtained from 35 patients in the danusertib 6-hour infusion schedule and in 8 patients in the 3-hour schedule. Samples from pa-



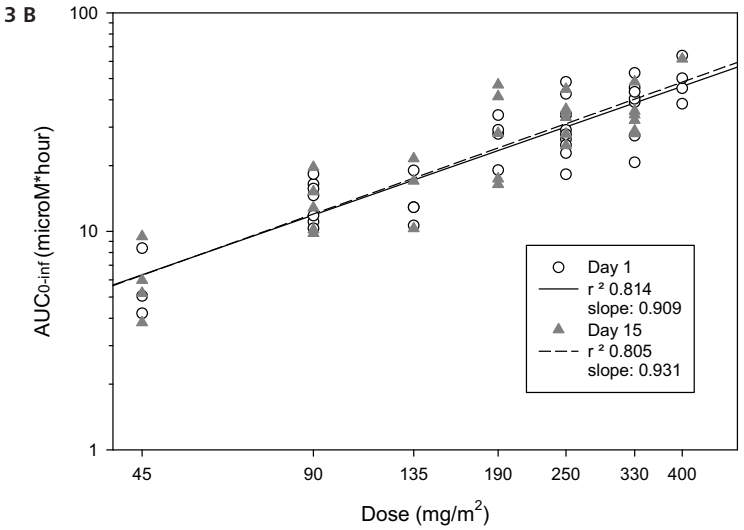


Fig. 3 A Day 1 individual AUC0-∞ of PHA-739358 vs. dose after 6-hour infusion of PHA-739358. Slope t-test: Day 1: t = 1.277, NS (df = 37)

Fig. 3 B Day 1 and day 15 individual AUC0-∞ of PHA-739358 vs. dose after 6-hour infusion of PHA-739358. Slope t-test: Day 1: t = 1.277, NS (df = 37)

tients at the 90 and 135 mg/m^2 dose levels (6h-ivS) were not evaluated because no phosphorylated histone H3 (pH3) was appreciated by Western blot (WB). In total, 30 patients had both pre and post treatment evaluable samples by IHC. By both WB (data not shown) and IHC (Figure 5) more than 80% pH3 inhibition was observed starting from the 190 mg/m^2 dose level (6h-ivS). These results are in agreement with the literature. Ex-

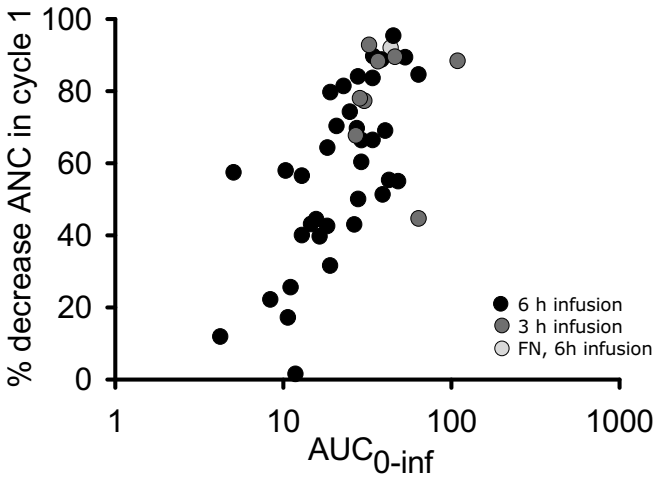


Fig. 4 Correlation between the percentage of decrease in neutrophil count, nadir vs baseline, during cycle 1 and the plasma AUC of PHA-739358.

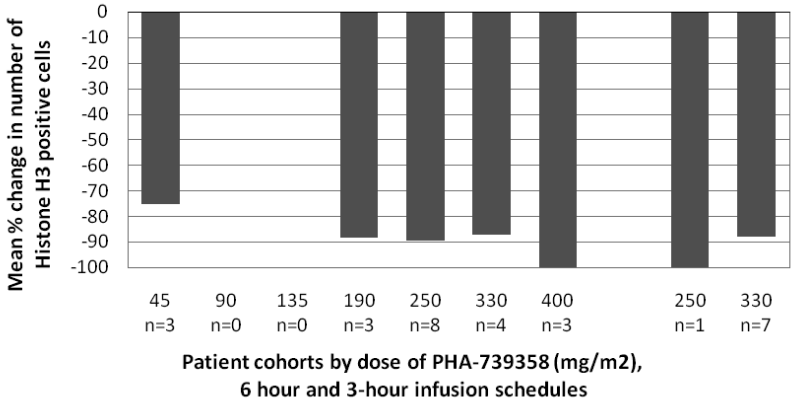


Fig. 5 Mean % change in number of Histone H3 positive cells by immunohisto-chemistry in skin biopsies; PHA-739358 on-treatment compared to pre-treatment.

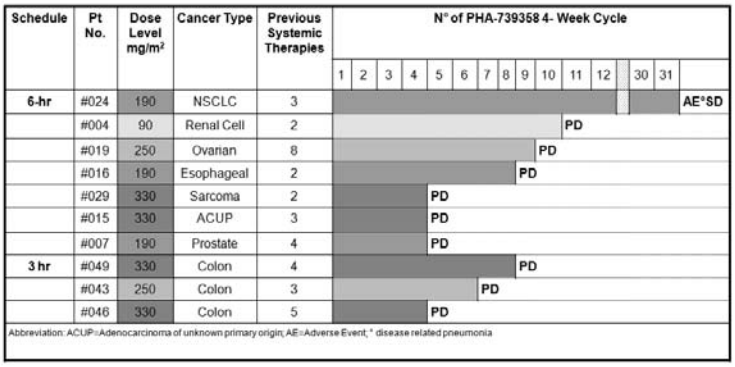


Fig. 6 Characteristics of patients with stable disease.

ploratory analysis of correlation between pH3 and clinical outcome was not conducted due to limited patient numbers.

Blood Pressure Mediators in Plasma

In the absence of a clear modulation of blood pressure mediator levels and blood pressure increase in two patients (one with hypertension during infusion and one without) (data not shown), these markers were not further explored and the blood sampling for this purpose was stopped.

Anti tumor activity

There were no complete or partial responses. An overall disease control rate (DCR) of 20.0% (6/30 patients) was observed in the 6h-ivS. DCR was 37.5% in the 3h-ivS (3/8 patients). Disease stabilization lasting >6 months was seen in 4 patients in the 6h-ivS, and in 1 patient in the 3h-ivS. One patient with progressive non small cell lung cancer prior to study entry, showed disease stabilization for over 2 years on the 6h schedule (Figure 6).

Discussion

In this study we demonstrate that treatment with the pan-aurora (A, B, and C) kinase inhibitor danusertib is well tolerated.

As aurora kinases are key regulators of mitosis, inhibition of their activity is likely to result in effects on bone marrow and other organ systems. Indeed neutropenia is dose

limiting in this and other studies with aurora kinase inhibitors.[17-27] Neutropenia is generally uncomplicated and of short duration. Limited non-hematological toxicity, such as mucositis, nausea, vomiting, diarrhea or alopecia is seen.

Recently aurora-A knockout mice were generated.[28,29] The aurora-A null mice died early during embryonic development, supporting the fact that aurora-A has a critical role in normal mitosis. Disturbingly, aurora-A heterozygote mice showed an increased incidence of malignancy.[28] The long-term effects of aurora kinase inhibition in man remain unknown.

Inhibition of pH3 more than 80%, indicating adequate aurora-B inhibition, was observed at dose levels ≥190 mg/m^2. This is in line with other publications.[10,12,14,21,25] However, since pH3 was inhibited in almost all patients, even in patients with clear tumor progression, the usefulness of this biomarker should be subject to exploration in future phase II and III studies. Other biomarkers like the number of mitotic cells in basal epithelium, FDG-PET, and dynamic-contrast enhanced magnetic resonance imaging are also being evaluated.[17,22,26,27,30]

Determining antitumor activity of danusertib was a secondary endpoint. Complete or partial responses were not observed. However, the overall disease control rate of 23.7% and long lasting disease stabilization (≥6 months) in some patients are indicative of antitumor activity and merit confirmation in a phase II study program.

Due to the limited patient numbers superiority or equivalence of the 3h or 6h schedule could not be concluded based on the PK results. The decision to recommend 330 mg/m^2 danusertib infused over 6 hours using the days 1, 8, 15 in a 28-day cycle schedule as the dose regimen for phase II investigations in solid tumors of is based on two observations. First, by shortening the infusion time to 3 hours, the dose intensity would have been lower that with the 6h-ivS (250 vs. 330 mg/m^2). Second, incidence and severity of toxicities was higher at the 330 mg/m^2 dose level when infusion time was shortened. Phase I studies investigating 24-hour infusion of danusertib are ongoing. danusertib also inhibits wild-type and mutated form of Abl, including the T315I mutant. A pilot phase II clinical study with the 6-h-IV schedule every 28 days is ongoing in patients with chronic myeloid leukemia (CML) relapsing on imatinib or other c-ABL therapy.[31,32] Preliminary results showed objective responses in 2 out of 7 CML patients with T315I mutations with an acceptable tolerability and safety profile.[33] Other cross-reactivities, including FGFRs, Ret and TrkA have been identified and could open additional venues for clinical development of danusertib.[10,11]

Currently many aurora-selective small-molecule inhibitors are undergoing preclinical and clinical studies. All have their individual advantages and disadvantages. MLN8054 was the first aurora kinase inhibitor with the advantage of oral administration. However, in phase I studies grade 3 somnolence was the main dose limiting toxicity, resulting from

binding of MLN8054 to the γ-aminobutyric acid α 1 benzodiazepine receptor.[17,26] MK-0457 is an intravenously administered aurora kinase inhibitor with positive off-target effects blocking the T315I-mutant BCR-ABL leading to clinical responses in 3 BCR-ABL dependent leukemia patients.[34] Danusertib, that inhibits all three aurora kinases, is also able to inhibit wild-type Abl as well as the most clinically frequent imatinib-resistant Abl mutants.[31]

The aurora kinase inhibitors have the advantage of not inducing alopecia and neurotoxicity related to other microtubular inhibitory agents. This can be taken into account when combining aurora kinase inhibitors with standard chemotherapy or targeted agents which will likely be part of future investigations.

In conclusion, danusertib administered in a 6h-ivS and 3h-ivS on days 1, 8, 15 of a 28 day cycle is safe and well tolerated. Based upon clinical endpoints, 330 mg/m^2 as 6h-ivS is the recommended dose for phase II studies.

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References

1. Kimura M, Kotani S, Hattori T, et al: Cell cycle-dependent expression and spindle pole localization of a novel human protein kinase, Aik, related to Aurora of Drosophila and yeast Ipl1. J Biol Chem 272:13766-13771, 1997.
2. Glover DM, Leibowitz MH, McLean DA, et al: Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. Cell 81:95-105, 1995.
3. Roghi C, Giet R, Uzbekov R, et al: The Xenopus protein kinase pEg2 associates with the centrosome in a cell cycle-dependent manner, binds to the spindle microtubules and is involved in bipolar mitotic spindle assembly. J Cell Sci 111 (Pt 5):557-572, 1998.
4. Tatsuka M, Katayama H, Ota T, et al: Multinuclearity and increased ploidy caused by overexpression of the aurora- and Ipl1-like midbody-associated protein mitotic kinase in human cancer cells. Cancer Res 58:4811-4816, 1998.
5. Terada Y, Tatsuka M, Suzuki F, et al: AIM-1: a mammalian midbody-associated protein required for cytokinesis. EMBO J 17:667-676, 1998.
6. Kimura M, Matsuda Y, Yoshioka T, et al: Cell cycle-dependent expression and centrosome localization of a third human aurora/Ipl1-related protein kinase, AIK3. J Biol Chem 274:7334-7340, 1999.
7. Sasai K, Katayama H, Stenoien DL, et al: Aurora-C kinase is a novel chromosomal passenger protein that can complement Aurora-B kinase function in mitotic cells. Cell Motil Cytoskeleton 59:249-263, 2004.
8. Warner SL, Gray PJ, Von Hoff DD: Tubulin-associated drug targets: Aurora kinases, Polo-like kinases, and others. Semin Oncol 33:436-448, 2006.

9. Carvajal RD, Tse A, Schwartz GK: Aurora kinases: new targets for cancer therapy. Clin Cancer Res 12:6869-6875, 2006.
10. Carpinelli P, Ceruti R, Giorgini ML, et al: PHA-739358, a potent inhibitor of Aurora kinases with a selective target inhibition profile relevant to cancer. Mol Cancer Ther 6:3158-3168, 2007.
11. Fancelli D, Moll J, Varasi M, et al: 1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazoles: identification of a potent Aurora kinase inhibitor with a favorable antitumor kinase inhibition profile. J Med Chem 49:7247-7251, 2006.
12. Carpinelli P, Moll J: Aurora kinase inhibitors: identification and preclinical validation of their biomarkers. Expert Opin Ther Targets 12:69-80, 2008.
13. Therasse P, Arbuck SG, Eisenhauer EA, et al: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216, 2000.
14. Soncini C, Carpinelli P, Gianellini L, et al: PHA-680632, a novel Aurora kinase inhibitor with potent antitumoral activity. Clin Cancer Res 12:4080-4089, 2006.
15. Camidge DR, Pemberton MN, Growcott JW, et al: Assessing proliferation, cell-cycle arrest and apoptotic end points in human buccal punch biopsies for use as pharmacodynamic biomarkers in drug development. Br J Cancer 93:208-215, 2005.
16. Agresti A: Categorical Data Analysis. 2002 , John Wiley & Sons, Inc, Hoboken, New Jersey.
17. Dees E, Infante JR, Cohen RB, et al: Phase I and pharmacokinetic study of MLN8054, a selective inhibitor of Aurora A kinase. Eur J Cancer 6:12S, 2008 (suppl; abstr 281).
18. Renshaw S, Patnaik A, Gordon M, et al: A phase I two arm trial of AS703569 (R763), an orally available aurora kinase inhibitor, in subjects with solid tumors: preliminary results. J Clin Oncol 25:18s, 2007 (suppl; abstr 14130).
19. Rubin EH, Shapiro GI, Stein MN, et al: A phase I clinical and pharmacokinetic (PK) trial of the aurora kinase (AK) inhibitor MK-0457 in cancer patients. J Clin Oncol 24:18s, 2006 (suppl; abstr 3009).
20. Schellens JH, Boss D, Witteveen PO, et al: Phase I and pharmacological study of the novel aurora kinase inhibitor AZD1152. J Clin Oncol 24:18s, 2006 (suppl; abstr 3008).
21. Foran JM, Ravandi F, O'Brien SM, et al: Phase I and pharmacodynamic trial of AT9283, an aurora kinase inhibitor, in patients with refractory leukemia. J Clin Oncol 26:18s, 2008 (suppl; abstr 2518).
22. Schöffski P, Dumez H, Jones SF, et al: Preliminary results of a Phase I accelerated dose-escalation, pharmacokinetic and pharmacodynamic study of PF-03814735, an oral Aurora kinase A and B inhibitor, in patients with advanced solid tumors. Eur J Cancer 6:12S, 2008 (suppl; abstr 282).
23. Robert F, Hurwitz H, Uronis H, et al: Phase 1 trial of SNS-314, a novel selective inhibitor of Aurora kinases A, B, and C, in advanced solid tumor patients. Eur J Cancer 6:12S, 2008 (suppl; abstr 283).
24. Cohen RB, Jones SF, von Mehren M, et al: Phase I study of the pan aurora kinases (AKs) inhibitor PHA-739358 administered as a 24 h infusion without/with G-CSF in a 14-day cycle in patients with advanced solid tumors. J Clin Oncol 26:18s, 2008 (suppl; abstr 2520).
25. Plummer ER, Calvert H, Arkenau H, et al: A dose-escalation and pharmacodynamic study of AT9283 in patients with refractory solid tumours. J Clin Oncol 26:18s, 2008 (suppl; abstr 2519).
26. Cervantes A, Macarulla T, Rosello S, et al: MLN8054, a selective inhibitor of Aurora A kinase: final results of a phase I clinical trial. Eur J Cancer 6:12S, 2008 (suppl; abstr 279).
27. Infante J, Dees EC, Cohen RB, et al: Phase I study of the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of MLN8237, a selective Aurora A kinase inhibitor, in the United States. Eur J Cancer 6:12S, 2008 (suppl; abstr 280).
28. Lu LY, Wood JL, Ye L, et al: Aurora a is essential for early embryonic development and tumor suppression. J Biol Chem 2008.
29. Sasai K, Parant JM, Brandt ME, et al: Targeted disruption of Aurora A causes abnormal mitotic spindle assembly, chromosome misalignment and embryonic lethality. Oncogene 27:4122-4127, 2008.
30. Cervantes A, Macarulla T, Rosello S, et al: MLN8054, a selective inhibitor of Aurora A kinase: final results of a phase I clinical trial. Eur J Cancer 6:12S (suppl; abstr 279) 6:90, 2008.
31. Tentler J, Pierce ELB, Serkova NJ, et al: ENMD-2076 exerts antiangiogenic and antiproliferative activity against human colorectal cancer (CRC) xenograft models. Eur J Cancer 6:12S, 2008 (suppl; abstr 284).
32. Modugno M, Casale E, Soncini C, et al: Crystal structure of the T315I Abl mutant in complex with the aurora kinases inhibitor PHA-739358. Cancer Res 67:7987-7990, 2007.
33. Gontarewicz A, Balabanov S, Keller G, et al: Simultaneous targeting of Aurora kinases and Bcr-Abl kinase by the small molecule inhibitor PHA-739358 is effective against imatinib-resistant BCR-ABL mutations including T315I. Blood 111:4355-4364, 2008.
34. Paquette RL, Shah NP, Sawyers CL, et al: PHA-739358, an Aurora Kinase Inhibitor, Induces Clinical Responses in Chronic Myeloid Leukemia Harboring T315I Mutations of BCR-ABL. Blood 110:11, 2007 (abstr 1030).
35. Giles FJ, Cortes J, Jones D, et al: MK-0457, a novel kinase inhibitor, is active in patients with chronic myeloid leukemia or acute lymphocytic leukemia with the T315I BCR-ABL mutation. Blood 109:500-502, 2007.

Appendix:

Pharmaceutical preparation of danusertib

Danusertib was supplied as a 10 mg/mL concentrate for solution for infusion, dosed at 15 mL/vial. One vial contained 150 mg of Danusertib in 5% dextrose solution adjusted to pH 5 with hydrochloric acid or sodium hydroxide (the hydrochloride salt of Danusertib is formed in situ during sterile aqueous solution manufacture)..

Pharmacokinetic Evaluation

Concentrations of danusertib were determined in human plasma by liquid chromatography-tandem mass spectrometry techniques (LC-MS-MS) following plasma protein precipitation in the 96-well plate format..

Briefly, plasma samples were extracted with acetonitrile containing a stable labeled internal standard. After centrifugation, the organic phase was transferred into a fresh 96-well and dried under nitrogen gas at 37°C. The residue was re-constituted with 15 mM ammonium formate buffer solution pH 3.0 and then aliquots injected into the LC-MS-MS system. Detection was by positive ion electrospray tandem mass spectrometry using Multiple Reaction Monitoring (MRM) following reversed phase chromatography on a Bonus RP column. The method was fully validated within the calibration range of 0.5-500 ng/ml.

Phospho histone H3 analysis in skin

Skin biopsies for biomarker analysis were performed on day 1 of the first cycle, before start and 10 minutes before end of the infusion. Skin samples were fixed in formalin, paraffin embedded and then analyzed for the phospho histone H3 staining by immunohistochemistry (IHC). The sections were stained with an anti-phosphorylated histone H3 (pH3) Ser10 polyclonal antibody (Upstate Biotechnology, NY, USA) and then counterstained with hematoxylin. A median number of linear dermis evaluated was 16 mm/sample. For every patient, the number of pH3 positive cells every 2 mm of dermis at pretreatment and at the end of treatment was defined as well as the % of change versus pretreatment. Biopsies were processed for immunohistochemistry (IHC), using an anti-phospho histone H3 antibody.