A phase I study of danusertib (PHA-739358) in adult patients with accelerated or blastic phase chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia resistant or intolerant to imatinib and/or other second generation c-ABL therapy

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ABSTRACT

Danusertib is a pan-aurora kinase inhibitor with potent activity against Abl kinase including the gatekeeper T315I mutant. A phase 1 dose escalation study of danusertib was conducted in patients with accelerated or blastic phase chronic myeloid leukemia or Philadelphia chromosome-positive acute lymphoblastic leukemia. Two dosing schedules were studied: schedule A, in which danusertib was given by 3-hour intravenous infusion daily for 7 consecutive days (days 1-7) in a 14-day cycle, and schedule B, in which the danusertib was given by 3-hour intravenous infusion daily for 14 consecutive days (days 1-14) in a 21-day cycle. A total of 37 patients were treated, 29 with schedule A and eight with schedule B. The recommended phase 2 dose for schedule A was 180 mg/m^2. Enrollment to schedule B was stopped early because of logistical problems with the frequency of infusions. Febrile neutropenia and mucositis were dose-limiting toxicities in schedule A. Four patients with T315I ABL kinase mutation, all treated with schedule A, responded. Danusertib has an acceptable toxicity profile and is active in patients with Bcr-Abl-associated advanced hematologic malignancies. This study was registered with the European Clinical Trails Data Base (EudraCT number 2007-004070-18).

Introduction

The aurora kinases are serine/threonine kinases that are evolutionarily conserved in eukaryotes and are essential for coordinated cell division.[1,2] There are three members of the family, aurora A, B and C, and they all play crucial roles in centrosome maturation, mitotic spindle formation, chromosome segregation and cytokinesis.[3-5] While their carboxy-terminal catalytic domains share a high level of sequence homology, the N terminus sequences are very diverse. Physiologically, the highest levels of aurora kinase expressions are seen in the lympho-hematopoietic and gastrointestinal systems and in the testes. Aurora kinases are overexpressed in multiple human cancers and are thus targets for inhibition of tumor cell division.[6,7]

Acquired resistance to tyrosine kinase inhibitors (TKI) targeting BCR-ABL is frequently associated with mutations in the ABL kinase domain leading to therapeutic failure in chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph$^+$ ALL).[8-11] The T315I mutant is resistant to first- and second-generation TKI; ponatinib, a third-generation TKI, is the only approved TKI with clinical activity against the T315I mutant.[12] Despite the central role that ABL kinase domain mutations play in the emergence of resistance to TKI, mechanisms other than mutations also exist for emergence of resistance to TKI.[13]

Danusertib is a small molecule ATP competitive pan-aurora kinase inhibitor that inhibits the catalytic domain of aurora kinases with IC_{50} of 13, 79 and 61 nM against aurora A, B and C kinases, respectively.[14] In addition, danusertib inhibits ABL (IC_{50} 25 nM) including the T315I and other mutants.[15] Danusertib is active against a broad panel of BCR-ABL-positive and -negative human cell lines including murine BAF3 cells expressing wild-type human BCR-ABL or with T315I and other clinically relevant mutations at sub-micromolar concentrations. Proliferation of CD34$^+$ cells derived from patients with imatinib-resistant CML in blast phase with (IC_{50} 19 nM) or without (IC_{50} 9nM) T315I mutation is suppressed by danusertib at nanomolar concentrations. Crystal structure resolution of T315I mutant ABL in the presence of danusertib shows that danusertib binds to the ATP binding pocket of the enzyme in the active conformation and the presence of the bulky isoleucine residue of T315I does not hinder binding.[16]

In phase 1 trials of danusertib in advanced solid tumors the dose-limiting toxicity (DLT) was neutropenia lasting more than 7 days when danusertib was administered without gran-

 doi:10.3324/haematol.2014.115279
The online version of this article has a Supplementary Appendix.
Manuscript received on September 9, 2014. Manuscript accepted April 13, 2015.
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ulocyte colony-stimulating factor.[17,18] This DLT was expected considering the cell cycle effect of danusertib. Phosphorylation of the histone H3 tail is critical for mitosis to progress and aurora B kinase is a primary mitotic kinase responsible for the phosphorylation on serine 10.[19] Thus, loss of histone H3 phosphorylation can be used as a pharmacodynamic biomarker of aurora kinase inhibition. In a phase 1 trial in solid tumors, diminished phosphorylation of histone H3 was demonstrated in concomitant skin biopsies, confirming the pharmacodynamic effects of danusertib.

Based on the *in vitro* activity of danusertib against ABL kinase, including most TKI-resistant mutants, we conducted a phase 1 study of danusertib in adult patients with advanced CML in accelerated or blastic phase (CML-AP or CML-BP) or Ph+ ALL, resistant or intolerant to imatinib and/or second-generation TKI.

Methods

The primary objective was to determine the maximum tolerated dose (MTD), and DLT of danusertib administered as a 3-h intravenous infusion daily for 7 consecutive days (days 1 to 7) of a 14-day cycle (schedule A) or daily for 14 consecutive days of a 21-day cycle (schedule B) in adult patients with advanced CML-AP or CML-BP or Ph+ ALL, resistant or intolerant to imatinib and/or second-generation ABL kinase inhibitors. The secondary objectives included an analysis of the safety, pharmacokinetics, pharmacodynamics and clinical activity of danusertib.

Initially all patients were to be assigned to schedule A, until the MTD was determined. Thereafter patients were to be allotted to schedule B to define the MTD with this schedule. Subjects provided informed consent and were enrolled in the institutionally approved protocol.

Dose escalation and modifications

Dose escalation was based on a standard "3+3" design. A modified Fibonacci design was followed for the dose escalation scheme. Intra-patient dose escalation was allowed after at least two cycles at the initially assigned dose. Dose modifications for hematologic and non-hematologic toxicities are summarized in the *Online Supplementary Data* (dose modifications). Toxicities were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 3.0. All patients treated were evaluable for toxicity.

Dose-limiting toxicity definitions

DLT were defined as events occurring in cycle 1, attributable to the test drug, and included prolonged myelosuppression (cellularity <10%) in the absence of marrow blasts, grade ≥3 non-hematologic toxicity except for nausea/vomiting/diarrhea controlled with supportive care, a significant decrease of left ventricular ejection fraction, and uncontrolled hypertension. Other hematologic toxicities were not included in the definition of DLT.

Eligibility

Adults (≥ 18 years) with CML-AP, CML-BP,[20] or Ph+ ALL, resistant or intolerant to imatinib and one second-generation Abl-kinase inhibitor were eligible. In France, patients had to be resistant/intolerant to both nilotinib and dasatinib in addition to imatinib. Additional criteria for inclusion were Performance Status ≤2, normal blood pressure, and adequate hepatic and renal function. Important reasons for exclusion were active central nervous system leukemia, uncontrolled infection, cardiac ejection fraction <40% by transthoracic echocardiography, uncontrolled cardiac issues e.g. uncontrolled arrhythmias, myocardial infarction or thromboembolic event within the preceding 6 months. Exclusion because of QTc >450 msec and risk factors for *torsade-de-pointes* were added as amendments.

Response definitions

Hematologic response was defined according to Talpaz *et al.*[21] while cytogenetic and molecular responses were defined according to Baccarani *et al.*[20] Briefly, a complete hematologic response required normalization of the white blood cell count with an absolute neutrophil count ≥1x10^9/L and platelet count ≥100x10^9/L, absence of blasts and promyelocytes and no extramedullary involvement. The same criteria were applied for response to be counted as no evidence of leukemia, but in this case the platelet count was allowed to be ≥20x10^9/L and ≤100x10^9/L and the absolute neutrophil count ≥0.5x10^9/L and ≤1x10^9/L. Cytogenetic response evaluation required assessment of at least 20 metaphases (from a bone marrow sample) and complete cytogenetic response required absence of Ph+ metaphases while the presence of >0 to ≤35% Ph+ metaphases defined a partial cytogenetic response.

Pharmacokinetic and pharmacodynamic studies

Pharmacokinetic samples were collected at pre-specified time points and plasma concentrations of danusertib were measured by validated liquid chromatography–tandem mass spectrometry techniques.

Peripheral blood mononuclear cells were isolated using a Ficoll-Hypaque gradient centrifugation procedure and analyzed for histone H3 (HH3) and Crkl phosphorylation status by western blot[15] followed by densitometric analysis. Each sample was normalized to the level of total histone H3. The following antibodies were used: phospho-H3-Ser10 (Upstate), histone-H3 (Abcam) and phospho-Crkl (Cell Signaling Technology).

Results

A total of 37 patients were treated using the two schedules, 29 with schedule A and eight with schedule B. Of the 37 patients, 22 had CML (7 CML-AP and 15 CML-BP) and 15 had Ph+ ALL. The demographic details of the treated patients are summarized in Table 1.

Schedule A

Twenty-nine patients (19 males and 10 females) with a median age of 51 years (range, 23-81 years) were enrolled in this cohort. Sixteen patients (55%) had advanced CML [7 CML-AP and 9 CML-BP (myeloid BP=8)] and 13 (45%) had Ph+ ALL. Seventeen (59%) patients carried a T315I mutation (Ph+ ALL=11, CML-AP=3, CML-BP=3), four (14%) had a kinase mutation other than T315I (F486S, F317L, Y253H and E255V, 1 patient each), and no mutation data were available for seven patients. Four (14%) patients were intolerant to imatinib and/or a second-generation TKI while 25 (86%) were resistant. The median number of prior therapies including TKI, monoclonal antibodies, alpha interferon and cytotoxic agents was three (range, 1-7). Nine (31%) patients had undergone prior stem cell transplantation (Ph+ ALL=5, CML-BP=3, CML-AP=1).

Toxicities. Patients were accrued to five dose levels: 90 mg/m^2 (n=7), 120 mg/m^2 (n=4), 150 mg/m^2 (n=6), 180 mg/m^2 (n=6) and 200 mg/m^2 (n=6) (Table 2). The 29 patients treated with schedule A received a total of 103 2-

week cycles. The median number of cycles per patient was two (range, 1-28 cycles). Twenty-one patients continued treatment beyond cycle 1. Three patients, one at the 90 mg/m^2 dose level (with progressive pneumonia) and two at the 200 mg/m^2 dose level (with disease progression), were not evaluable for DLT because of removal from study due to serious adverse events unrelated to the study therapy or death. One patient at the 90 mg/m^2 dose level and two at the 200 mg/m^2 level experienced DLT. The DLT for the patient treated at 90 mg/m^2 was grade 3 syncope, while that for the two patients treated at 200 mg/m^2 was mucositis, one each of grade 3 and 4 mucositis (one also had grade 3 bullous dermatitis). Thus the 180 mg/m^2 dose level was expanded to six patients and in the absence of any DLT this dose level was identified as the MTD and the recommended dose for phase 2. Two patients were off study after completing cycle 1 for disease progression.

The most frequent drug-related events (occurring in ≥20% of patients) were diarrhea (62%), nausea (59%), anemia and hypomagnesemia (35% each), headache, hypokalemia and vomiting (28% each), hypoalbuminemia and mucosal inflammation (24% each), elevation of aspartate transaminase, hypocalcemia, hypophosphatemia and stomatitis (21% each). CTC grade 3-4 drug-related events were reported by 19 (66%) patients, with the most frequent being anemia (n=6, 21%), febrile neutropenia (n=5, 17%), diarrhea and thrombocytopenia (n=4, 14%). No infusion site-related reactions were reported. Table 3 summarizes all treatment-related and clinically significant toxicities reported for schedule A.

Nine deaths (Ph$^+$ ALL=6, CML-BP=3) on study were reported, none of them considered related to the study drug: four were due to disease progression, and one each due to multi-organ failure secondary to sepsis, multi-organ failure with cardiac arrest, respiratory failure, pneumonia leading to cardiopulmonary arrest and aspergillus pneumonitis.

Responses. Responses were observed in four (20%) of the 20 evaluable patients, all carrying the T315I BCR-ABL mutation. Briefly, a Ph$^+$ ALL patient (patient n. 009 in Table 4) (previously treated with imatinib and dasatinib in combination with chemotherapy and allogeneic transplantation), received danusertib 120 mg/m^2, and achieved a complete hematologic response (after cycle 4), complete cytogenetic response (after cycle 7) and undetectable transcript levels (complete molecular response) after seven cycles of treatment. The complete molecular response was maintained until cycle 9 (changed to a major molecular response, which lasted until cycle 27). The complete cytogenetic response was confirmed until cycle 16 (not assessed beyond that). Another patient with CML-AP achieved a complete hematologic response after cycle 1 which lasted until the end of cycle 5. A third patient with Ph$^+$ ALL achieved a partial cytogenetic response after cycle 3 that lasted until the end of cycle 6. Both patients had disease progression after the sixth cycle of treatment. One CML-BP patient, treated at a dose level of 150 mg/m^2, achieved a complete cytogenetic response without recovery of counts after two cycles of danusertib. This patient then underwent stem cell transplantation. The summary of these four patients is presented in Table 4.

Pharmacokinetics. A pharmacokinetic evaluation of danusertib was carried out using a non-compartmental approach with the aid of Winnonlin software (version 3.1, Pharsight Inc.). Cycle 1 day 1 and day 7 mean ± SD plasma maximal concentration and daily area-under-the-curve (AUC) parameters of danusertib are reported in Table 5. Day 1 and day 7 mean plasma concentrations of danusert-

Table 1. Baseline demographics and patients' characteristics.

Characteristics	Schedule A 3h-IV on D1-7 q2w (n=29) N. (%)	Schedule B 3h-IV on D1-14 q3w (n=8) N. (%)	All patients (n=37) N. (%)
Age (years)			
Median(range)	51.2(23-81)	46.0 (23-67)	48(23-81)
Gender			
Female	10 (34.5)	1 (12.5)	11 (29.7)
Male	19 (65.5)	7 (87.5)	26 (70.3)
Performance status (ECOG)			
0	4 (13.8)	1 (12.5)	5 (13.5)
1	15 (51.7)	4 (50.0)	19 (51.4)
2	10 (34.5)	3 (37.5)	13 (35.1)
Diagnosis			
Ph$^+$ ALL	13 (44.8)	2 (25.0)	15 (40.5)
CML	16 (55.2)	6 (75.0)	22 (59.5)
CML-AP	7 (24.1)	6 (75.0)	7 (18.9)
CML-BP	9 (31.0)	-	15 (40.5)
Myeloid blast phase	8 (27.5)	6 (75)	14 (37.8)
Lymphoid blast phase	1 (3.5)	-	1 (2.7)
BCR-ABL mutation status			
T315I	17 (58.6)	3 (37.5)	20 (54.1)
Others*	4 (13.9)	1(12.5)**	5 (13.5)
Status at study entry			
Resistant to imatinib and/or ≥ one 2nd generation c-ABL therapy	25 (86.2)	8 (100)	33 (89.2)
Intolerant to imatinib and/or 2ndgeneration c-ABL therapy	4 (13.8)	-	4 (10.8)
Prior therapies			
Median n. (range)	3 (1-7)	2 (1-7)	
Tyrosine kinase inhibitors	29 (100.0)	8 (100.0)	37 (100.0)
Imatinib	28 (96.6)	7 (87.5)	35 (94.6)
Dasatinib	9 (31.0)	3 (37.5)	12 (32.4)
Nilotinib	4 (13.8)	1 (12.5)	5 (13.5)
Bosutinib	8 (27.6)	1 (12.5)	9 (24.3)
Stem-cell transplant	9 (31.0)	3 (37.5)	12 (32.4)

*ALL: acute lymphoblastic leukemia; CML: chronic myeloid leukemia; CML-AP: chronic myeloid leukemia in accelerated phase; CML-BP: chronic myeloid leukemia in blast phase ECOG: Eastern Cooperative Oncology Group; NA: not assessed; NE: not evaluable. *including F486S, F317L, Y253H, E255V (1 patient each), ** mutation data missing for four patients in schedule B.*

Table 2. Dose escalation scheme, treatment duration, first cycle DLT (schedule A).

Dose level (mg/m^2)	N. of patients	Total n. of cycles	Dose limiting toxicities n.	event(s)
90	7	21	1	G3 syncope
120	4	35	0	
150	6	15	0	
180 (RP2D)	6	17	0	
200	6	15	2	G4 stomatitis & G3 bullous dermatitis (n=1) G3 mucosal inflammation (n=1)
Any dose	29	103	3	

G: grade of toxicity by CTC criteria; RP2D: recommended phase 2 dose.

ib are plotted in Figure 1. After day 1 and day 7 doses the interpatient variability of maximal concentration and daily AUC of danusertib was of the order of 30-50%. In the dose range of 90 - 200 mg/m^2 mean ± SD daily AUC increased from 6.78±1.84 μM·h to 15.9±8.42 μM·h after the first administration and from 6.40±2.34 μM·h to 15.3±5.64 μM·h on day 7. Across doses, negligible accumulation ratios of maximal concentration (1.3) and daily AUC (1.1) were observed.

Pharmacodynamics. Samples from only 12 patients for histone H3 and 11 for Crkl were suitable for pharmacodynamic analysis. The rest of the samples were either not collected or showed degradation. A densitometric analysis of phosphorylated proteins at baseline and at end of infusion was performed and the results are reported in Figure 2. In most cases a better inhibition of histone H3 phosphorylation was observed (≥50% inhibition in 67% of patients treated at doses ≥150 mg/m^2) compared to Crkl phosphorylation. Nevertheless a significant (≥50%) inhibition of Crkl phosphorylation was observed in four of the 11 evaluable samples. Despite some variability and the limited number of patients, the effect is consistent with danusertib's activity of aurora kinase and BCR-ABL inhibition. Unfortunately none of the four responders had optimal samples for pharmacodynamic analysis.

Schedule B

In schedule A several patients had decreases in circulating blasts during the days of danusertib infusion followed by increases during the weeks off therapy. Schedule B was designed to explore whether administration of danusertib for 14 consecutive days every 3 weeks would result in better disease control. Eight patients (myeloid-BP=6 and Ph$^+$ ALL=2) were enrolled to schedule B (7 patients at 120 mg/m^2 and 1 patient at 140 mg/m^2): seven patients were resistant to imatinib and/or at least one second-generation TKI; three patients (CML-BP=2, Ph$^+$ ALL=1) had T315I mutations and three patients had received prior SCT (CML-BP=2, Ph$^+$ ALL=1). Accrual to this dose schedule was then stopped as the frequency of intravenous administrations was impractical for most patients. Three patients were taken off study prior to completion of cycle 1 because of disease progression (2 patients) or respiratory failure with pneumonia (1 patient). These eight patients received a total of 11 3-week cycles of therapy. No first cycle DLT was recorded in any of the eight treated patients and no recommended phase 2 dose was identified with this schedule of treatment. Two patients were evaluable for efficacy and none had a protocol-defined response. Four deaths (2 disease progression, 1 pneumonia and 1 sepsis/multi-organ failure) occurred on study and

Table 3. Clinically significant adverse events related to study treatment by dose group (schedule A).

Event	CTC grade	Assigned dose level (mg/m^2/day) 90 (n=7) N.	120 (n=4) N.	150 (n=6) N.	180 (n=6) N.	200 (n=6) N.	Any dose (n=29) N.	%
Any term	Any grade	6	4	6	6	6	28	96.6
Diarrhea	1-2	3	2	3	4	2	14	48.3
	3-4	-	1	-	1	2	4	13.8
Nausea	1-2	1	3	3	5	5	17	58.6
	3-4	-	-	-	-	-	-	-
Vomiting	1-2	1	2	-	2	2	7	24.1
	3-4	-	-	-	-	1	1	3.4
Headache	1-2	1	3	1	1	2	8	27.6
	3-4	-	-	-	-	-	-	-
Constipation	1-2	1	1	-	-	1	3	10.3
	3-4	-	-	-	-	-	-	-
Mucosal inflammation NOS	1-2	1	2	1	-	1	5	17.2
	3-4	-	-	-	1	1	2	6.9
Stomatitis	1-2	1	-	-	1	2	4	13.8
	3-4	1	-	-	-	1	2	6.9
Febrile neutropenia	1-2	-	-	-	-	-	-	-
	3-4	1	1	2	-	1	5	17.2
Creatinine increased	1-2	3	-	-	-	1	4	13.8
	3-4	-	-	-	-	-	-	-
Hypertension	1-2	1	-	1	-	2	4	13.8
	3-4	-	-	-	-	-	-	-
Hypotension	1-2	-	1	-	1	-	2	6.9
	3-4	-	-	-	1	-	1	3.4
Hyponatremia	1-2	-	-	-	-	2	2	6.9
	3-4	-	-	-	-	1	1	3.4
Peripheral edema	1-2	-	-	1	1	1	3	10.3
	3-4	-	-	-	-	-	-	-
AST increased	1-2	2	-	1	1	1	5	17.2
	3-4	-	-	-	-	1	1	3.4
Cytolytic hepatitis	1-2	-	-	-	-	-	-	-
	3-4	-	1	-	-	1	2	6.9
Mental status changes	1-2	-	-	-	1		1	3.4
	3-4	-	-	-	-	1	1	3.4
Acute respiratory distress syndrome	1-2	-	-	-	-	-	-	-
	3-4	-	-	-	-	1	1	3.4
Bullous dermatitis	1-2	-	-	-	-	-	-	-
	3-4	-	-	-	-	1	1	3.4
Tumour lysis syndrome	1-2	-	-	-	-	-	-	-
	3-4	-	-	-	1	-	1	3.4

Note: toxicities presented according to worst CTC grade across all cycles. AST: aspartate transaminase

Table 4. Details of response (schedule A).

Pt. n.	Disease	Prior systemic therapies	BCR-ABL mutation	Dose level (mg/m^2)	Total n. of cycles	Best response to danusertib hematologic	cytogenetic	molecular
001	CML-AP	I, N , AKI (MK-0457), D , MKI/ARA C, HHT	T315I	90	6	Complete		
002	PH$^+$ALL	Hyper-CVAD, I, Mesna/CTX+DX+MTX+Ara C,D, JAK2 inhibitor	T315I	90	9	Minor	Partial	
009	PH$^+$ALL	CTX+VCR+DX+MTX+Ara C; I, D, N; allo-SCT, DLI	T135I	120	28	Complete	Complete	Complete
012	CML-BP	I, D, Ara C	T315I	150	2*	Minor	Complete	NA*

*AKI: aurora kinase inhibitor; Ara C: cytarabine; D: dasatinib; HU: hydroxyurea; I: imatinib; HHT: hemoharringtonine; N: nilotinib; CTX: cyclophosphamide; DX: doxorubicin; MKI: multi kinase inhibitor; MTX: methotrexate; VCR: vincristine; allo-SCT: allogeneic stem cell transplant; DLI: donor lymphocyte infusion; NA: not assessed. * This patient proceeded to stem cell transplant after cycle 2.*

none was drug-related. The most frequent (frequency ≥25%) drug-related events were constipation (4 cases, 50%), diarrhea (3 cases, 38%), mucositis, nausea and thrombocytopenia (2 cases each, 25%). CTCAE grade 3 drug-related events were reported by three patients (38%) all treated at the 120 mg/m^2 dose level: fatigue and thrombocytopenia (1 patient), febrile neutropenia and asthenia (1 patient), and mucosal inflammation (1 patient).

Pharmacokinetic samples after repeated administrations of study drug were available for four patients treated at 120 mg/m^2. Maximal concentration (mean ± SD: 3.65 ± 3.23 μM) and daily AUC (10.3 μM·h, n=1) were similar to the corresponding figures on schedule A. No samples for biomarkers modulation were suitable for analysis in patients treated with schedule B.

Discussion

In this trial in which danusertib was administered as a single agent in an intermittent schedule, syncope and mucositis were DLT-defining events and the recommended dose for phase 2 was 180 mg/m^2 danusertib administered intravenously over 3 h for 7 consecutive days every 2 weeks. With this schedule the dose intensity at the recommended dose for phase 2 is at least twice the dose intensity administered to patients with solid tumors (630

Table 5. Plasma pharmacokinetic parameters (mean ±SD) of danusertib (schedule A).

Dose	Day 1		Day 7	
(mg/m^2)	C_{max} (μM)	$AUC_{0\text{-}24}$(μM·h)	C_{max} (μM)	$AUC_{0\text{-}24}$(μM·h)
90	1.71 ±0.927 (n=7)	6.78 ±1.84 (n=4)	1.85±1.02 (n=5)	6.40±2.34 (n=5)
120	2.95 ±1.18 (n=4)	9.60 ±1.94 (n=3)	4.07±1.16 (n=4)	13.6±2.50 (n=3)
150	3.41 ±0.447 (n=4)	11.4 ±2.28 (n=3)	4.37±1.60 (n=5)	13.3±4.39 (n=4)
180	4.62 ±1.61 (n=6)	7.78 (n=1)	2.01±1.79 (n=4)	5.44 (n=1)
200	3.60 ±2.68 (n=6)	15.9 ±8.42 (n=3)	4.26±2.38 (n=4)	15.3±5.64 (n=4)

C_{max}: maximal concentration, $AUC_{0\text{-}24}$: daily area under concentration versus *time curve.*

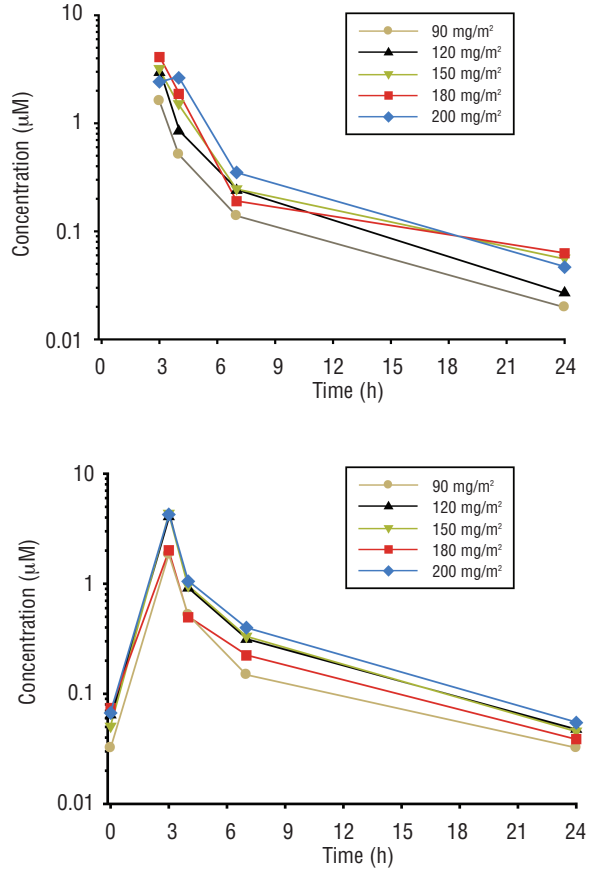

Figure 1. Mean plasma concentrations (μM) of danusertib on day 1 (upper panel) and day 7 (lower panel) of cycle 1 after a 3-h infusion (schedule A).

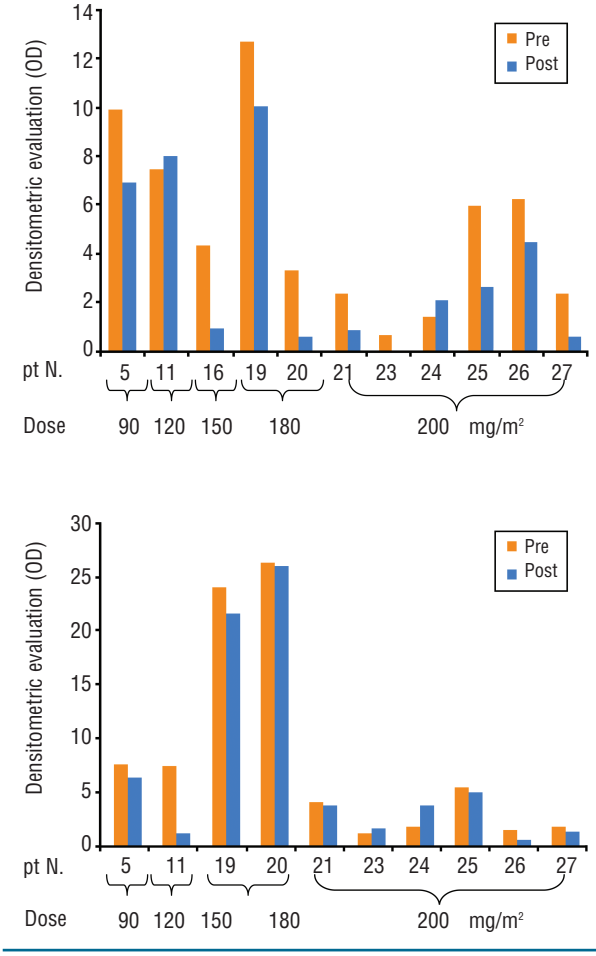

Figure 2. Histone H3 (upper panel) and Crkl phosphorylation (lower panel) in peripheral blood mononucleated cells (schedule A).

mg/m²/week *versus* 250 mg/m²/week in solid tumors). The DLT events are among the side-effects expected from the cell cycle effect of danusertib on proliferating cells. In other trials of danusertib in solid tumors, neutropenia and neutropenia complicated by infection were identified as DLT.[17,18] In one study in which danusertib was co-administered with granulocyte colony-stimulating factor in a single 24-h infusion every 14 days, renal toxicity (namely grade 1-2 creatinine increase) emerged as a potential DLT.[18] No treatment-related renal toxicity was observed in our study.

In the current study, responses were seen in four (20%) patients treated with schedule A, all carrying the T315I mutation. Responses were reported at three different dose levels (90, 120 and 150 mg/m²) and the best response was observed at 120 mg/m². Three of these four patients were heavily pre-treated with multiple chemotherapeutic regimens in addition to other TKI. These three responding patients continued therapy for 6, 9 and 28 cycles, respectively. The fourth patient was not evaluable for response duration because he received a stem cell transplant after the second cycle while still with a complete cytogenetic response.

However, rebound of circulating blast count was observed at all dose levels after transient decreases in peripheral blood blasts in the majority of non-responding patients in the week off therapy. This was the rationale for exploring a longer exposure schedule (schedule B). Schedule B was, however, considered cumbersome to deliver because of the frequency of administration.

Responses in the form of clinically significant disease stabilizations have been seen in solid tumor trials with danusertib. In a phase 2 trial that enrolled patients with previously treated metastatic colorectal cancer, 3/31 patients had stable disease for 6-8.5 months. Similarly, in a phase 2 randomized trial in patients with castration-resistant prostate cancer progressing after docetaxel-based chemotherapy, exploring two different schedules of administration of danusertib, 12 of 58 evaluable patients had clinically relevant disease stabilization with neither arm proving better.[22]

Aurora kinase inhibitors other than danusertib are also active against ABL kinase. MK-0457 is a pan aurora kinase inhibitor, active against BCR-ABL-positive ALL cells with or without T315I mutations in a pre-clinical model in which leukemia cells were grown in contact with stromal cells,[23] which has shown clinical activity in patients carrying such mutations.[24] In a phase I/II study, 8/18 patients with CML and a T315I mutation had a hematologic response with MK-0457; gastrointestinal toxicities, including mucositis, were relatively common. Two non-aurora kinase inhibitors, namely ponatinib and omacetaxine, are currently approved for the treatment of chronic or advanced phase CML resistant or intolerant to two or more TKI and have significant activity in the presence of resistant mutations, including T315I.[12,25,26] Lastly, barasertib (AZD1152) is another aurora kinase B inhibitor that has been studied in myeloid leukemias, including in elderly patients with AML, either as a single agent or in combination with low-dose cytarabine, with evidence of promising clinical activity.[27,28]

In pre-clinical models danusertib has also demonstrated additive or synergistic activity with sorafenib (hepatocellular carcinoma)[29] and imatinib (CML).[30] Thus, exploring combinations of danusertib with kinase inhibitors (FLT3, ABL, MEK etc.) in the context of relevant mutations as well as with standard chemotherapy would be of interest.

Surrogate skin biopsies have been used in solid tumor trials to show reductions in histone H3-positive cells in the epidermis as evidence of target inhibition by danusertib.[18] Although limited numbers of optimal samples were available, the pharmacodynamic studies in our trial indicated target inhibition, which was better in the form of reduced histone H3 phosphorylation but also to a lesser extent with reduced Crkl phosphorylation in leukemic cells.

In summary, danusertib has shown an acceptable toxicity profile, expected from its mechanism of action, and demonstrated early promising activity in advanced hematologic malignancies associated with BCR-ABL fusion and resistance to TKI. The extent of exposure to danusertib increased with dose and was time-independent. Its activity against T315I mutations makes it a particularly promising agent and development of oral formulations should substantially help its clinical development. Based on its aurora kinase inhibitory activity, danusertib could be active in other hematologic malignancies and studies of its combination with targeted agents or chemotherapeutic drugs need to be pursued.

Acknowledgments

This work was supported by research funding provided by Nerviano Medical Sciences.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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