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ORIGINAL ARTICLE

Phase 1 study of MLN8054, a selective inhibitor of Aurora A kinase in patients with advanced solid tumors

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Abstract
Purpose Aurora A kinase is critical in assembly and function of the mitotic spindle. It is overexpressed in various tumor types and implicated in oncogenesis and tumor progression. This trial evaluated the dose-limiting toxicities (DLTs) and maximum tolerated dose (MTD) of MLN8054, a selective small-molecule inhibitor of Aurora A kinase.
Methods In this first-in-human, dose-escalation study, MLN8054 was given orally for 7, 14, or 21 days followed by a 14-day treatment-free period. Escalating cohorts of 3–6 patients with advanced solid tumors were treated until DLT was seen in $\geq$2 patients in a cohort. Serial blood samples were collected for pharmacokinetics and skin biopsies were collected for pharmacodynamics.
Results Sixty-one patients received 5, 10, 20, 30, or 40 mg once daily for 7 days; 25, 35, 45, or 55 mg/day in four divided doses (QID) for 7 days; or 55, 60, 70, or 80 mg/day plus methylphenidate or modafinil with daytime doses (QID/M) for 7–21 days. DLTs of reversible grade 3 benzodiazepine-like effects defined the estimated MTD of 60 mg QID/M for 14 days. MLN8054 was absorbed rapidly, exposure was dose proportional, and terminal half-life was 30–40 h. Three patients had stable disease for >6 cycles.
Conclusions MLN8054 dosing for up to 14 days of a 28-day cycle was feasible. Reversible somnolence was dose limiting and prevented achievement of plasma concentrations predicted necessary for target modulation. A recommended dose for investigation in phase 2 trials was not established. A second-generation Aurora A kinase inhibitor is in development.

Keywords MLN8054 · Aurora A kinase · Dose-limiting toxicity · Pharmacokinetics · Pharmacodynamics

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Introduction

The Aurora kinases are a family of serine/threonine protein kinases. Three isoforms of Aurora kinase exist (Aurora A, B, and C), each with distinct activities. Aurora A and B have critical roles in the normal progression of cells through mitosis, whereas Aurora C activity is largely restricted to meiosis. Aurora A kinase localizes to centrosomes and proximal mitotic spindles [1], where it regulates centrosome maturation/separation, the G2-M transition, formation of mitotic spindle poles and spindles, and chromosome alignment and separation [2–5]. Increased Aurora A kinase expression results in oncogenic transformation in preclinical models [6–9] and has been correlated

with decreased survival in patients with solid tumors [10, 11]. Aurora A kinase is amplified and overexpressed in many solid tumors and hematological malignancies [12–16]. Consequently, Aurora A kinase is an attractive target for anticancer treatment [17].

MLN8054 (Fig. 1; Millennium, the Takeda Oncology Company) is an orally active small molecule that selectively inhibits Aurora A kinase [18]. MLN8054 induces severe mitotic defects, including delayed progression through mitosis, formation of abnormal mitotic spindles and misaligned chromosomes, and chromosome segregation defects [18, 19]. MLN8054 led to decreased tumor proliferation in models of human cancer grown in cell culture and antitumor activity in human tumor xenografts including colon, prostate, and lung cancer models [18]. The greatest efficacy was seen with once or twice daily dosing for 21 days in mice, suggesting that prolonged target inhibition results in maximal antitumor activity. In preclinical toxicology studies, dose-limiting toxicities (DLTs) were myelosuppression and gastrointestinal toxicity, and MLN8054 demonstrated high-affinity binding to the alpha-1 subunit of the GABA-A receptor (Data on file, Millennium). Preclinical pharmacokinetic/pharmacodynamic analyses suggest antitumor activity is dose dependent, and maintenance of plasma concentrations of ~2,000 nM for 8–12 h per day is required for efficacy in human tumor xenografts grown in mice [20].

Hepatic biotransformation of MLN8054 was studied in vitro using human liver S9 fractions (Data on file, Millennium). Glucuronidation of the carboxylate moiety of MLN8054 to an acyl glucuronide was the predominant mechanism of biotransformation, Hydroxylation of the azepine moiety of MLN8054 was the major phase 1 biotransformation pathway. Glucuronidation was mediated by UGT1 and UGT2 and hydroxylation by CYP1A2, 2C9, 2C19, 2D6, and 3A4.

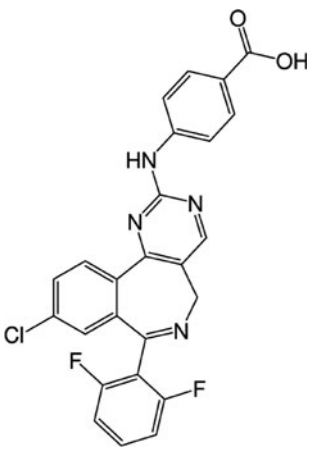

Fig. 1 Chemical structure of MLN8054 (Reprinted from Manfredi et al. [18]). Copyright 2007 National Academy of Sciences, USA

This phase 1 study was conducted to: (1) determine the dose-limiting toxicity (DLT) and maximum tolerated dose of MLN8054 when given orally for 7, 14, or 21 days, followed by a 14-day recovery period, the latter thought to be necessary based on neutropenia results from preclinical toxicology studies; (2) describe the pharmacokinetics of MLN8054 from serial blood samples; (3) evaluate the relationship between MLN8054 exposure and inhibition of Aurora A kinase in skin basal epithelial cells; and (4) describe any antitumor activity of MLN8054.

Materials and methods

Design

This open-label phase 1 study (NCT00249301) was conducted at 3 centers in the United States between October 19, 2005, and January 25, 2008. The study followed the principles of the Declaration of Helsinki. The protocol was reviewed and approved by the institutional review board at each clinical center. Each patient provided informed written consent prior to enrollment.

Eligibility

Patients with a solid tumor malignancy refractory to conventional treatment or for whom no standard treatment existed were candidates for this study. Patients were required to be ≥18 years of age and to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, expected survival greater than 3 months from study enrollment, and adequate hematologic, renal, and hepatic function. Prior cytotoxic chemotherapy was limited to no more than 4 regimens, and prior radiation therapy must have included less than 25% of the hematopoietically active bone marrow. Patients were ineligible if they had central nervous system metastases, had undergone peripheral blood stem cell or bone marrow transplantation, or had prior gastrointestinal surgery or conditions that would impair absorption.

Dose escalation

The dose escalation scheme is shown in Table 1. In animal studies, dogs were the most sensitive species to MLN8054 and the highest non-severely toxic dose in dogs was 20 mg/m^2/day. One quarter of this dose (5 mg/m^2/day) was chosen as the starting dose for this first-in-human study. Patients were enrolled in escalating dose cohorts of 3–6 patients each; if 1 of 3 patients had a DLT, the cohort was expanded to 6 patients. If 0–1 of 6 patients experienced a DLT, then dose escalation continued. If ≥2 of 3–6 patients

Table 1 Dose-escalation scheme and dose-limiting toxicities

Schedule/total daily dose	Dosing days/ cycle days	Divided dose (mg)	No. of patients	No. with DLT	Dose-limiting toxicity	Grade	Day	Dose action[a]
Single daily dosing for 7 days								
QD-7D 5 mg	7/21	–	4	0	–	–	–	–
QD-7D 10 mg	7/21	–	4	0	–	–	–	–
QD-7D 20 mg	7/21	–	3	0	–	–	–	–
QD-7D 30 mg	7/21	–	6	1	Somnolence	2	2	Reduced
QD-7D 40 mg	7/21	–	4	2	Somnolence	3	1	Reduced
					Somnolence	3	1	Discontinued
Divided dosing for 7 days								
QID-7D 25 mg	7/21	5/5/5/10	6	1	Somnolence	3	5	Discontinued
QID-7D 35 mg	7/21	5/5/5/20	3	0	–	–	–	–
QID-7D 45 mg	7/21	5/5/5/30	3	0	–	–	–	–
QID-7D 55 mg[b]	7/21	10/10/10/25	4	2	Somnolence	2	3	Reduced, held
					Somnolence	3	5	Reduced
Divided dosing with Methylphenidate or Modafinil for 7, 14, or 21 days[c]								
QID/M-7D 55 mg	7/21	10/10/10/25	3	0	–	–	–	–
QID/M-7D 60 mg	7/21	10/10/10/30	6	1	Somnolence	2	7	Reduced, held
QID/M-7D 70 mg	7/21	15/15/15/25	3	0	–	–	–	–
QID/M-7D 80 mg	7/21	15/15/25/25	3	1	Cognitive/hallucination	3/3	5/5	Reduced
QID/M-14D 70 mg	14/28	15/15/15/25	2	2	Somnolence	2	1	Reduced, held
					Fatigue/confusion/cognitive	3/3/3	4/4/4	Reduced
QID/M-14D 60 mg	14/28	10/10/10/30	3	0	–	–	–	–
QID/M-21D 60 mg	21/35	10/10/10/30	4	2	Somnolence	3	2	Held
					Somnolence/cognitive/confusion	3/3/3	2/2/2	Reduced, held

[a] Reduced, the next dose (in the same cycle) was reduced due to the toxicity; held, one or more doses were held until the toxicity abated

[b] Two patients in the QID-7D 55-mg cohort received the sympathomimetic agent, modafinil, for fatigue or somnolence

[c] Methylphenidate or modafinil was added to daytime doses to reduce central nervous system effects such as somnolence

experienced a DLT, then the MTD had been surpassed, and a lower dose level or alternate schedule was explored. Dose-escalation decisions incorporated real-time assessment of systemic drug exposure as well as toxicity experience and utilized the factors of 2 pharmacokinetically guided dose-escalation method [21]. Adverse events (AEs) were defined by CTCAE version 3.0 [22]. A DLT was defined as any of the following during cycle 1: grade 4 neutropenia lasting more than 7 days or associated with fever; grade 4 thrombocytopenia; grade 3 or greater nausea or diarrhea that persisted despite the use of optimal antiemetic or anti-diarrheal therapy; any other grade 3 or greater non-hematologic AE except arthralgia/myalgia or brief (<1 week) fatigue; or any drug-related AE requiring dose interruption or delay of more than one week.

Patients took MLN8054 orally on an empty stomach, with nothing by mouth 2 h before and 1 h after each dose except prescribed medications and water. The initial dosing regimen was once daily for 7 days (QD-7D), with 14-day breaks (21-day cycles). Due to somnolence with the QD regimen, the protocol was amended to include divided four-times-daily (QID-7D) dosing, with the highest dose at bedtime. The QID administration was designed to minimize daytime sedation (which was not observed in mice) and maximize exposure (supported by PK computer modeling) to potentially therapeutic concentrations of the compound [23]. In later QID cohorts, an oral psychostimulant (methylphenidate or modafinil) was added to daytime doses (QID/M-7D) to further mitigate somnolence. Additional cohorts received extended-duration QID/M dosing for 14–21 days per cycle (QID/M-14D and QID/M-21D), with 14-day breaks (28- and 35-day cycles, respectively).

Adverse event information was collected throughout the study. Safety assessments were based on evaluation of AEs and serious AEs (SAEs), including their potential relationship to the study medication; physical examination; monitoring of clinically significant laboratory tests, including hematologic parameters, liver function tests, and renal function tests; and evaluation of serial electrocardiograms.

Pharmacokinetics

Blood samples for pharmacokinetic analyses were drawn once at baseline (day 1 predose), serially on day 1 (QD dosing only—0.5-, 1-, 1.5-, 2-, 3-, 4-, 6-, 8-, 10-, and 24-h postdose) and day 7 (QD dosing—predose and 0.5-, 1-, 1.5-, 2-, 3-, 4-, 6-, 8-, 10-, and 24-h postdose; QID dosing—before the second daily dose and 0.5-, 1-, 1.5-, 2-, 3-, 4-, and 6-h postdose); and once daily on days 8–12.

Pharmacodynamics

For patients in the QD cohorts, serial 3-mm skin punch biopsies for pharmacodynamic analyses were obtained in a majority of patients ($n = 52$) at baseline and again 6 and 24 h after the first dose of cycle 1. For patients in the QID cohorts, skin punch biopsies were obtained at baseline and on day 7 before and 2 h after the second daily dose of MLN8054. The purpose of these biopsies was to detect inhibition of Aurora A kinase in proliferating basal epithelial cells as measured by accumulation of mitotic cells. Immunofluorescence analysis was performed on skin sections, using two mitotic markers, pHisH3 (Cell Signalling Technologies, Danvers, MA) and MPM2 (Cell Signalling Technologies, Danvers, MA). Skin sections were mounted with DAPI Vectashield Hard Set Mounting Medium (Vector Laboratories, Burlingame, CA). The mitotic index was determined by counting the number of mitotic cells per millimeter of the basal epithelial layer.

Before receiving the first dose of MLN8054, patients underwent disease evaluation including physical examination, computed tomography and/or magnetic resonance imaging, and tumor markers when applicable. Evaluations were repeated after every 2 cycles of MLN8054, and disease status was categorized using standard Response Evaluation Criteria in Solid Tumors (RECIST) guidelines [24]. Patients who had stable disease or a partial response continued treatment until there was evidence of disease progression or unacceptable treatment-related toxicity. Patients who tolerated the first cycle of treatment with MLN8054 were allowed to increase the dose of MLN8054 treatment in subsequent cycles of treatment if the higher dose had been found to be tolerable in a subsequent cohort.

Statistical analysis

The safety population included all patients who received at least one dose of study drug, while the DLT population included all patients who received study drug at the assigned dose level and had sufficient follow-up to determine whether a DLT occurred. Descriptive statistics are reported for baseline values, safety, pharmacokinetics, and pharmacodynamics.

Results

Patient characteristics

A total of 61 patients were treated in 16 dose cohorts (see Table 1) and were evaluable for toxicity. Demographics and baseline characteristics for the safety population are shown in Table 2. The majority of patients were men (61%) and median age was 60 (range 24–80 years). All patients had a performance status of 0 (54%) or 1 (46%). The most common primary diagnoses were colorectal cancer (36%), lung cancer (15%), genitourinary tumors (13%), and sarcoma (13%). Most patients had been heavily pretreated, with 79% having received 3 or more courses of prior treatment before study entry.

Reasons for discontinuation were progressive disease in 39 patients (64%), symptomatic deterioration in 9 (15%), AE in 5 (8%), withdrawal of consent in 3 (5%), death of 1 (2%) due to cardiac arrest unrelated to MLN8054, and other reasons in 4 (7%). The median number of MLN8054

Table 2 Patient demographics and baseline characteristics ($n = 61$)

Characteristic	All patients
Male/female, *n* (%)	37 (61)/24 (39)
Median age (range, years)	60 (24–80)
Race/ethnicity (*n*, %)	
White	48 (79)
Black	10 (16)
Hispanic	2 (3)
Asian/Pacific Islander	1 (2)
Primary tumor type, *n* (%)	
Colorectal	22 (36)
Lung	9 (15)
Genitourinary	6 (10)
Sarcoma	7 (11)
Pancreatic	4 (7)
Gynecologic	3 (5)
Breast	3 (5)
Head and neck	2 (3)
Liver	2 (3)
Esophageal	1 (2)
Gallbladder	1 (2)
Melanoma	1 (2)
ECOG performance status score	
0	33 (54)
1	28 (46)
No. of prior therapies, *n* (%)	
0	2 (3)
1–2	11 (18)
3+	48 (79)

ECOG Eastern cooperative oncology group

treatment cycles was 2 (range 1–14). Patients received 98% of all expected doses overall.

Dose-limiting toxicity

Although 60 unique patients were enrolled in the study, 1 patient was enrolled twice and was separately evaluated for safety as a member of both the QD-7D 40-mg cohort and the QID-7D 25-mg cohort, and thus 61 patients were evaluable for safety. A total of 59 patients (97%) were evaluable for DLT; the other 2 patients discontinued treatment during cycle 1, before DLT could be evaluated. One patient in the QD-7D 5-mg cohort discontinued due to hospitalization for renal failure and hypovolemia unrelated to MLN8054, and one patient in the QID/M-21D 60-mg cohort discontinued due to hospitalization for spine fracture unrelated to MLN8054.

Table 1 summarizes the observed DLTs by cohort. Somnolence, which resolved in all but one patient, was the only DLT for MLN8054 given without methylphenidate or modafinil. The onset of somnolence and its severity generally were correlated with dose and C_{max}. Because somnolence was thought to be related to C_{max}, the dosing schedule was changed from daily to QID dosing in an effort to lower peak plasma concentrations and allow further dose escalation. However, dose-limiting somnolence was seen at both QD-7D dosing (1 of 6 patients at 30 mg and 2 of 4 patients at 40 mg) and QID-7D dosing (1 of 6 patients at 25 mg and 2 of 4 patients at 55 mg).

The addition of methylphenidate (e.g. 5 mg oral dose) or modafinil repeated as needed with the three daytime MLN8054 doses allowed further dose escalation. Dose limiting but reversible somnolence was seen in 1 of 6 patients in the QID/M-7D 60-mg cohort, and grade 3 cognitive disorder and hallucination were experienced by 1 of 3 patients in the QID/M-7D 80-mg cohort. The QID/M-7D 80-mg cohort was not expanded to further define the MTD using the 7-day schedule because all three patients at this level experienced grade $\geq$2 CNS effects, and the investigator consensus was to evaluate longer treatment schedules if possible. With 14-day dosing, DLTs of reversible somnolence or other central nervous system effects (fatigue, confusional state, and cognitive disorder) were seen in 2 of 2 patients at QID/M-14D 70 mg. Because these events were seen during the first week of a planned 2-week treatment, subsequent patients were enrolled to a QID/M-14D 60-mg cohort. None of the 3 patients in this cohort had a DLT. Using a QID schedule with 10 mg administered 3 times during the day and 30 mg at night, the total daily dose of 60 mg was generally tolerable over a 7- to 14-day schedule.

Of the 4 patients who were enrolled to a QID/M-21D 60-mg cohort, 2 had a DLT. Both patients had a DLT of somnolence and 1 of the patients also had DLTs of cognitive disorder and confusion. Therefore, no additional cohorts were enrolled to 21-day dosing regimens.

Thus, the estimated MTD of MLN8054 was 60 mg divided QID for 7–14 days, given with methylphenidate or modafinil as needed with the daytime doses to manage somnolence.

Adverse events

All 61 patients (100%) were treated and were evaluable for safety. Table 3 summarizes the most frequent drug-related adverse events, which included somnolence, fatigue, confusion, nausea, and vomiting. Forty-seven patients (77%) experienced drug-related somnolence, with 11 (18%) experiencing grade 3 somnolence. Many of the patients in this study received at least one other medication that could have contributed to somnolence; 37 (61%) received an opioid analgesic, 15 (25%) received a benzodiazepine or other anxiolytic, and 12 (20%) received a hypnotic or sedative agent.

Among the 11 patients with dose-limiting somnolence related to MLN8054 across all dose levels, concomitant use of opioid medication was reported in 8 patients. Opioid use was reported frequently in many patients enrolled to this study however, and the frequency of somnolence was comparable in patients who were or were not receiving concomitant opioids. Moreover, dose-limiting (CTC grade 3) somnolence was reported in 2 patients without concomitant treatment with opioids or other sedating medications who received the highest MLN8054 dose levels

Table 3 Drug-related adverse events: any grade in $\geq$10% of patients or grade $\geq$3 in any patient ($n = 61$)

Adverse event	Any grade	Grade 3[a]
Any drug-related adverse event	55 (90)	13 (21)
Somnolence	47 (77)	11 (18)
Fatigue	15 (25)	1 (2)
Confusion	11 (18)	4 (7)
Nausea	10 (16)	0 (0)
Anorexia	8 (13)	0 (0)
Dizziness	7 (11)	1 (2)
Vomiting	6 (10)	0 (0)
Asthenia	6 (10)	1 (2)
Abnormal coordination	5 (8)	1 (2)
Hyperglycemia	5 (8)	1 (2)
Muscular weakness	5 (8)	1 (2)
Increased blood alkaline phosphatase	4 (7)	1 (2)
Hyperbilirubinemia	3 (5)	1 (2)
Peripheral motor neuropathy	1 (2)	1 (2)
Hallucination	1 (2)	1 (2)

[a] No patient had a grade 4 or 5 drug-related adverse event

within the first days of dosing, so that further dose escalation was not feasible even in a population not receiving concomitant opioid medications.

Thirty-four patients (56%) had a grade ≥3 AE, including 13 patients (21%) with a drug-related grade 3 AE (Table 3); no patient had a drug-related grade 4 or 5 AE. No dose studied was associated with grade ≥3 mucositis or myelosuppression, predicted to be mechanistic effects associated with Aurora A kinase inhibition.

Nine patients (15%) died within 21 days of the last dose of MLN8054; none of these deaths were considered drug related.

Pharmacokinetics

Forty-eight patients (79%) had sufficient dosing and MLN8054 concentration–time data to estimate pharmacokinetics. Mean MLN8054 plasma concentration–time profiles for QD-7D doses are shown in Fig. 2a. Pharmacokinetic parameters for each cohort are summarized in Table 4. MLN8054 was absorbed over a short period, with T_{max} ranging from 1–4 h. Terminal elimination half-life ($t_{1/2}$) was 30–40 h. AUC_{0-24} h and C_{max} were roughly dose proportional with QD dosing, and the peak-to-trough ratio (C_{max}/C_{min}) for all dose levels was approximately 5. Only 1 patient in the QD-7D 30-mg cohort had a trough concentration on day 7 that was >2,000 nM, the predicted minimum concentration needed for inhibition of Aurora A kinase based on a tumor xenograft model [20]. The two patients with the highest day 1 C_{max} values among PK-evaluable patients enrolled in the QD dosing cohorts both experienced grade 3 somnolence.

The protocol was amended to implement QID-7D dosing to reduce C_{max}, thereby reducing somnolence, and to increase trough concentrations, thereby increasing the probability of inhibiting Aurora A kinase for a substantial period of time. Using this approach with total daily doses of 25, 35, 45, and 55 mg, the mean C_{max} values were 1,050, 1,966, 1,526, and 2,484 nM, respectively, and the mean trough concentrations after the second daily dose on day 7 were 720, 1,464, 1,139, and 1,802 nM, respectively. The peak-to-trough ratio was between 1.3 and 1.6 across these 4 cohorts, substantially lower than the ratio for QD dosing regimens. On average, drug concentrations for the highest dose, QID-7D 55 mg, were close to the target of 2,000 nM.

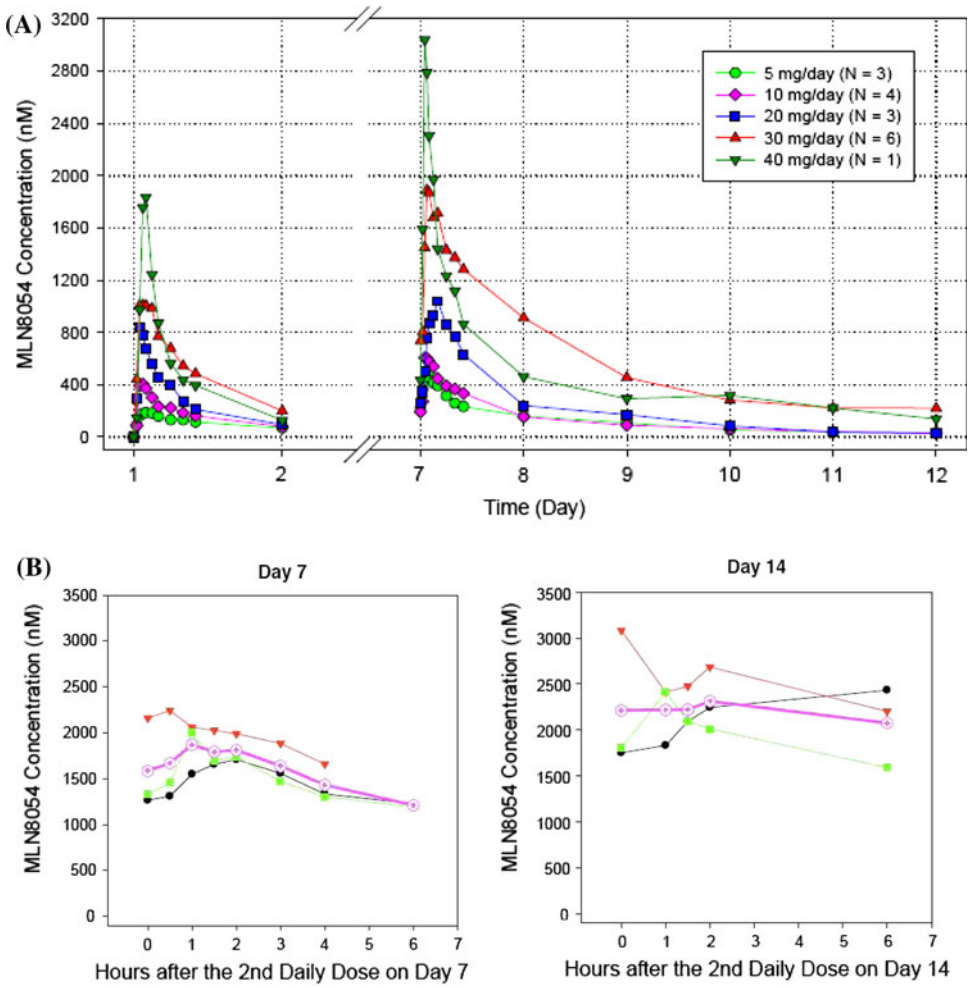

Fig. 2 MLN8054 plasma concentration–time profiles. **a** Mean values in the QD-7D cohorts; **b** individual patients in the QID/M-14D 60-mg cohort. *Open symbols* mean values; *closed symbols* individual data

Table 4 Summary of pharmacokinetic parameters at steady state

Cohort	No. of patients	No. with PK data	Mean (SD)				Geometric mean peak-to-trough ratio (C_{max}/C_{min})
			T_{max}, h	C_{max}, nM	C^{a}_{min}, nM	AUC_{0-24}, nM h	
QD-7D (day 7)							
5 mg	4	4	1.7 (0.3)	521 (441)	162 (154)	6,159 (5,469)	4.99
10 mg	4	4	3.8 (4.3)	724 (171)	154 (77)	7,526 (2,932)	5.10
20 mg	3	3	3.3 (1.2)	1,176 (364)	240 (113)	13,506 (2,704)	5.09
30 mg	6	6	3.0 (2.0)	2,362 (1,002)	912 (1,221)	28,215 (20,512)	4.72
40 mg	4	1	1.0[b]	3,040[b]	461[b]	24,184[b]	6.59[b]
QID-7D (day 7, dose 2)							
25 mg	6	6	2.5 (2.1)	1,050 (268)	720 (359)	4,850 (1,649)	1.58
35 mg	3	3	1.5 (1.3)	1,966 (585)	1,464 (301)	9,713 (2,294)	1.32
45 mg	3	3	0.8 (0.8)	1,526 (820)	1,139 (803)	7,553 (4,471)	1.45
55 mg	4	4	1.2 (1.0)	2,484 (921)	1,802 (846)	12,522 (5,785)	1.44
QID/M-7D (day 7, dose 2)							
55 mg	3	3	1.0 (0.0)	2,310 (1,447)	1,314 (919)	10,711 (7,759)	1.81
60 mg	6	6	1.3 (0.5)	1,947 (834)	1,092 (475)	8,375 (3,591)	1.80
70 mg	3	3	1.5 (0.0)	3,284 (878)	1,950 (204)	15,217 (3,381)	1.65
80 mg	3	3	1.0 (0.5)	2,129 (1,093)	1,674 (1,158)	10,109 (7,176)	1.35
QID/M-14D (day 14, dose 2)							
70 mg	2	0	–	–	–	–	–
60 mg	3	3	2.3 (3.2)	2,642 (381)	2,074 (436)	13,203 (1,779)	1.29
QID/M-21D (day 21, dose 2)							
21D 60 mg	4	1	1.0[b]	1,503[b]	945[b]	7,028[b]	1.59[b]

[a] C_{min} was measured 24 h after the daily dose in the QD cohorts and 6 h after the second dose in the QID cohorts

[b] Individual data are shown for outcomes with only one value recorded

Subsequent QID cohorts added oral methylphenidate or modafinil with each of the 3 daytime doses of MLN8054 to mitigate the impact of somnolence. Pharmacokinetic data from the QID/M-7D 70-mg cohort showed promise for maintaining a mean plasma MLN8054 concentration of 2,000 nM and a peak-to-trough ratio of 1.65 over the course of treatment.

When the duration of dosing was extended to QID/M-14D, both patients in the 70-mg cohort had a DLT and neither was evaluable for pharmacokinetics. The dose level was reduced to QID/M-14D 60 mg, and all 3 patients were evaluable for pharmacokinetics. Mean MLN8054 concentrations in these patients were close to 2,000 nM between the second and third doses on day 7 and were >2,000 nM between the second and third doses on day 14 (Fig. 2b).

Only 1 patient in the QID/M-21D 60-mg cohort was evaluable for pharmacokinetics on days 14 and 21, and MLN8054 concentrations in this patient were <2,000 nM between the second and third doses on each of these days.

Two of the 3 patients with the highest day 7 C_{max} values among PK-evaluable patients enrolled in the various QID dosing cohorts experienced grade 3 somnolence. One of these patients experienced grade 3 somnolence despite receiving methylphenidate. Thus, of the 48 patients with sufficient data to estimate pharmacokinetic parameters, 7 patients had trough MLN8054 concentrations that were >2,000 nM; namely 1 patient in the QD-7D 30-mg cohort, 1 patient in the QID-7D 55-mg cohort, 2 patients in the QID/M-7D 70-mg cohort, 1 patient in the QID/M-7D 80-mg cohort, and 2 patients in the QID/M-14D 60-mg cohort.

Pharmacodynamics

Skin biopsies were evaluable pre- and post-treatment in 52 patients. Although some patient skin samples had increased numbers of mitotic cells suggestive of Aurora A kinase inhibition after MLN8054 dosing, these increases generally

were slight and did not compellingly indicate Aurora A inhibition in any dose cohort. No relationship was observed between the pharmacodynamic parameters and either the MLN8054 dose or the MLN8054 pharmacokinetic parameters. Despite the fact that 7 patients had trough MLN8054 concentrations >2,000 nM, the skin biopsies in these patients did not provide significant evidence of Aurora A kinase inhibition.

Clinical responses

No complete or partial responses were seen. Nine patients (15%) had stable disease for at least 4 cycles, including 3 (5%) with stable disease for greater than 6 cycles. These included 1 patient in the QD-7D 5-mg cohort with colorectal cancer (8 cycles; 165 days), 1 patient in the QID/M-7D 55-mg cohort with extraskeletal chondrosarcoma (8 cycles; 129 days), and another patient in the QID/M-7D 55-mg cohort with spindle-cell sarcoma (12 cycles; 266 days).

Discussion

As critical regulators of the mitotic process that are frequently overexpressed in human tumors [25], the Aurora kinases have emerged as novel oncologic therapeutic targets. In addition to kinesin spindle (Eg5 motor protein) inhibitors, polo-like kinase inhibitors, and centromeric protein E (CENPE) inhibitors, Aurora kinase inhibitors represent a unique class of antimitotic agents without direct anti-tubulin properties [26]. Compared to taxanes and vinca alkaloids, Aurora kinase inhibitors may improve the therapeutic index by avoiding neurotoxicity and specifically targeting kinases that are only expressed in dividing cells.

Currently, several Aurora kinase inhibitors are in phase 1 or 2 testing [27]. Most of these are pan-Aurora inhibitors that inhibit isoforms A, B, and C. MLN8054 is the first selective Aurora A kinase inhibitor to enter clinical trials. Selective inhibition of Aurora A has potential advantages because it is amplified in many solid tumors and hematological malignancies [12–16]. Furthermore, selective Aurora A kinase inhibition may have a different toxicity profile and therapeutic index than pan-Aurora inhibitors based upon adverse events specific to inhibiting both Aurora A and Aurora B kinases simultaneously.

This is the first reported experience with an Aurora A kinase inhibitor in clinical testing. This novel oral compound was tolerated well at doses up to 60 mg/day in divided doses. Escalation was halted due to dose-limiting, reversible, benzodiazepine-like somnolence and neurocognitive changes, despite the addition of methylphenidate or modafinil in the higher dose cohorts. MLN8054 is structurally related to the benzodiazepines, and as such it has activity against the GABAA α1 receptor. Sedation had been expected from preclinical toxicological evaluation of MLN8054, although it was not anticipated that benzodiazepine-like central nervous system effects would be dose limiting. Because these neurocognitive side effects were thought to be partially dependent on peak plasma concentrations, once-daily dosing in the early cohorts was changed to divided daily doses in later cohorts in order to continue dose escalation. Although some patients with somnolence received concomitant treatment with opioids or other sedating medications, somnolence occurred with similar frequency in patients not receiving these medications. In addition, 3 of the patients who experienced dose-limiting somnolence (CTC grade 3) in cycle 1 did not receive concomitant treatment with opioids or other sedating medications. These findings indicate that the symptomatic somnolence seen in this study was due to MLN8054.

This phase 1 study also included pharmacokinetic analyses after the first dose in the once-daily cohorts and at steady state (the second dose on day 7) in the divided dosing cohorts. MLN8054 was absorbed rapidly, with peak concentrations at 1–4 h postdose and $t_{1/2}$ of 30–40 h. Drug exposures were roughly dose proportional in the QD dose range evaluated in this study. The peak-to-trough ratio was reduced from approximately 5 with once-daily dosing to 1.3–1.6 with QID dosing.

A steady-state concentration of 2,000 nM, the concentration estimated to be necessary for antitumor activity, was achieved in some but not all patients at the 60-mg dose, and in few patients in the other dose cohorts. Skin biopsies were evaluated for mitotic arrest in the basal epithelial cells at 24 h—a surrogate for inhibition of Aurora A. Although some samples had slightly increased numbers of mitotic cells, no clear relationship could be established with either the MLN8054 dose level or MLN8054 concentration across the range of exposures tolerable in this study. The most likely explanation is that prolonged biologically active exposures were not achieved in the patients tested in this study, as subsequent studies using the second-generation Aurora A kinase inhibitor MLN8237 have demonstrated dose-dependent pharmacodynamic activity in skin biopsies obtained at similar times using identical assays [28]. Furthermore, there was no consistent evidence of significant myelosuppression or mucositis, the expected anti-mitotic side effects of MLN8054. By contrast, other pan-Aurora kinase inhibitors have reported dose-related neutropenia that defined the MTD [29, 30]. No patient had a complete or partial response to the doses tested (5–80 mg per day) for 7, 14, or 21 days with 14-day breaks, although stable disease lasting

more than 6 cycles was observed in 3 patients; namely 1 at the lowest dose tested (QD-7D 5 mg) and 2 at the highest dose tested without methylphenidate or modafinil support (QID-7D 55 mg).

In summary, benzodiazepine-like effects, especially somnolence, were DLTs of MLN8054, despite QID dosing and the addition of an oral psychostimulant (methylphenidate or modafinil) with daytime doses. Benzodiazepine-like toxicities were expected, but it was not anticipated that their severity would prevent escalation to doses that provided sustained target plasma concentrations. At the doses studied, there was no evidence of antiproliferative effects such as myelosuppression, mucositis, or tumor response. Despite these limitations, MLN8054 did exhibit several favorable pharmaceutical features, including reliable absorption, dose-proportional systemic exposure, and sufficiently prolonged half-life to support a once-daily administration schedules. As an anticancer agent, the MLN8054 chemotype is an oral agent distinct from the other Aurora kinase inhibitors currently in development. Building on these results, MLN8237, a second-generation oral Aurora A kinase inhibitor, has been developed with structural modifications that are designed to improve the risk-to-benefit profile, particularly with regard to central nervous system effects. Clinical trials of MLN8237 are underway to evaluate the safety, pharmacokinetics, pharmacodynamics, and clinical response in patients with advanced tumors [31, 32].

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