Phase I Study of the Selective Aurora A Kinase Inhibitor MLN8054 in Patients with Advanced Solid Tumors: Safety, Pharmacokinetics, and Pharmacodynamics

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Abstract

This phase I trial examined the safety, pharmacokinetics, and pharmacodynamics of MLN8054, an oral, selective, small-molecule inhibitor of Aurora A kinase. Patients with advanced solid tumors received increasing doses of MLN8054 in 28-day cycles until dose-limiting toxicity (DLT) was seen in ≥2 of 3-6 patients in a cohort. For the 10-mg and 20-mg cohorts, treatment was administered once daily on days 1 to 5 and 8 to 12. Patients in later cohorts (25, 35, 45, 55, 60, 70, and 80 mg/day) were treated four times daily on days 1 to 14, with the largest dose at bedtime (QID-14D) to mitigate benzodiazepine-like effects possibly associated with peak plasma concentrations. Patients (n = 43) received a median of 1 cycle (range, 1–10). DLT of somnoience was first noted in the 20-mg cohort. Two DLTs of somnolence (n = 1) and transaminisits (n = 1) were seen at QID-14D 80 mg. Grade 2 oral mucositis (n = 1), predicted to be a mechanistic effect, was observed only at QID-14D 80 mg. MLN8054 exposure levels were roughly linear with dose; terminal half-life was 30 to 40 hours. Pharmacodynamic analyses of skin and tumor mitotic indices, mitotic cell chromosome alignment, and spindle bipolarity provided evidence of Aurora A inhibition. MLN8054 dosing for 10 to 14 days in 28-day cycles was feasible. Somnolence and transaminisits were DLTs. Pharmacodynamic analyses in mitotic cells of both skin and tumor provided proof of mechanism for Aurora A kinase inhibition. A more potent, selective, second-generation Aurora A kinase inhibitor, MLN8237, is in clinical development. Mol Cancer Ther; 9(10); 2844–52. ©2010 AACR.

Introduction

Aurora A kinase belongs to a highly conserved family of serine/threonine protein kinases that also includes Aurora B and Aurora C (1). The Aurora A gene maps to chromosomal region 20q13.2, which frequently is amplified and overexpressed in a diverse array of human cancers (2–4). Amplification of the Aurora A gene has been implicated in oncogenesis, tumor progression, and decreased survival (5, 6). Aurora A plays an essential role in the proper assembly and function of the mitotic spindle (7). Perturbation of Aurora A causes defects in centrosome separation, spindle pole organization, and chromosome alignment, leading to cell death (8).

MLN8054 (Fig. 1) is an oral, ATP-competitive, selective small-molecule inhibitor of Aurora A that was developed as a novel modality for therapeutic intervention in human cancers (8, 9). Aurora A inhibition leads to mitotic delays and severe chromosome congression and segregation defects, followed by cell death or arrest through the development of deleterious aneuploidy (8). In in vitro studies, MLN8054 inhibited the growth of a broad array of tumor cell lines (9), including cultured neuroblastoma cells through an apoptosis-dependent pathway (10). In animal studies, oral administration of MLN8054 dosed once or twice daily inhibited the growth of multiple human tumor xenografts grown in immunocompromised mice in a dose-dependent fashion (9). Continuous administration of MLN8054 via s.c. osmotic pump showed that the tumor mitotic index, a pharmacodynamic end point, and growth inhibition of colorectal carcinoma HCT-116 xenografts increased linearly with increasing plasma concentrations of drug and saturated at approximately 2,000 nmol/L (11).

Two concurrent phase I studies were conducted to evaluate the safety, pharmacokinetics, and pharmacodynamics of MLN8054. Here we report the findings from the second of these two studies, which began when the first study had already been initiated (12, 13); the study design for this trial therefore reflects informed decisions based on early observations from the first study.
The primary objective of this phase I study was to determine the dose-limiting toxicity (DLT) and the maximum tolerated dose (MTD) of MLN8054. Secondary objectives were to (a) describe the pharmacokinetics of MLN8054, (b) evaluate the pharmacodynamic relationship between MLN8054 exposure and inhibition of Aurora A kinase in skin basal epithelial cells and in tumor tissue, and (c) describe any antitumor activity of MLN8054. This study specifically aimed to assess the optimal dose and schedule of orally administered MLN8054, based on the safety profile and pharmacodynamic effects of Aurora A kinase inhibition in sequential tumor and skin biopsies.

Materials and Methods

This open-label phase I study (clinicaltrials.gov #NCT00652158) was conducted at two centers in Spain from April 2006 to March 2008. The study followed the principles of the Declaration of Helsinki. An institutional review board approved the study protocol for each site. Each patient provided informed written consent prior to enrollment.

Patients were eligible for the study if they had advanced solid tumors for which no curative or life-prolonging therapies existed. Study participants 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 >3 months from study enrollment, and adequate hematologic (absolute neutrophil count ≥1.5 × 10⁹/μL and platelet count ≥100 × 10⁹/μL), renal (serum creatinine ≤1.6 mg/dL and creatinine clearance ≥30 mL/minute), and hepatic function [bilirubin ≤1.25 times the upper limit of normal (ULN), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2 times the ULN, and alkaline phosphatase ≤2 times the ULN]. Prior cytotoxic chemotherapy was limited to no more than four regimens, and prior radiation therapy must have included <25% of the hematopoietically active bone marrow. Patients with central nervous system metastases and those who had undergone peripheral blood stem cell or bone marrow transplantation were not eligible to participate in this study.

The dose escalation scheme is shown in Table 1. Patients were enrolled in escalating dose cohorts of three to six patients each; if one of three patients had a DLT, enrollment continued in that cohort until at least two patients had a DLT or six patients were enrolled with no additional DLTs.

<table>
<thead>
<tr>
<th>Schedule/total daily dose</th>
<th>Divided dose, mg</th>
<th>No. of patients</th>
<th>No. with DLT</th>
<th>DLT</th>
<th>Grade</th>
<th>Day</th>
<th>Dose action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single daily dosing for 10 of 14 days with a 14-day break*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QD-10D 10 mg</td>
<td>—</td>
<td>3</td>
<td>0</td>
<td>Transaminitis</td>
<td>4</td>
<td>7</td>
<td>Discontinued</td>
</tr>
<tr>
<td>QD-10D 20 mg</td>
<td>—</td>
<td>6</td>
<td>1</td>
<td>Somnolence</td>
<td>3</td>
<td>7</td>
<td>Discontinued</td>
</tr>
<tr>
<td>Divided dosing for 14 days with a 14-day break†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QID-14D 25 mg</td>
<td>5/5/5/10</td>
<td>7</td>
<td>1</td>
<td>Somnolence</td>
<td>3</td>
<td>7</td>
<td>Reduced</td>
</tr>
<tr>
<td>QID-14D 35 mg</td>
<td>5/5/5/20</td>
<td>3</td>
<td>0</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QID-14D 45 mg</td>
<td>5/5/5/30</td>
<td>6</td>
<td>1</td>
<td>Somnolence</td>
<td>3</td>
<td>7</td>
<td>Reduced</td>
</tr>
<tr>
<td>QID-14D 55 mg</td>
<td>10/10/10/25</td>
<td>3</td>
<td>0</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QID-14D 60 mg</td>
<td>10/10/10/30</td>
<td>3</td>
<td>0</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QID-14D 70 mg</td>
<td>15/15/15/25</td>
<td>6</td>
<td>1</td>
<td>Hepatotoxicity</td>
<td>3</td>
<td>14</td>
<td>Reduced</td>
</tr>
<tr>
<td>QID-14D 80 mg</td>
<td>15/15/25/25</td>
<td>6</td>
<td>2</td>
<td>Somnolence</td>
<td>3</td>
<td>4</td>
<td>Held</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Transaminitis</td>
<td>3</td>
<td>15</td>
</tr>
</tbody>
</table>

*Administered 5 consecutive days/week for 2 weeks (i.e., days 1–5 and 8–12).
†Divided dosing was studied to reduce peak concentrations (reduce somnolence) and increase trough concentrations (increase on-target effects).
DLT. Adverse events (AE) were defined by Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. A DLT was defined as any of the following during the first cycle: (a) grade 4 neutropenia for >7 consecutive days, (b) grade 4 neutropenia with fever and/or infection, (c) grade 4 thrombocytopenia (platelets <25,000/mm³), (d) grade 3 nonhematologic AE except arthralgia/myalgia or brief (<1 week) fatigue, and (e) any drug-related AE requiring dose interruption or delay.

Patients took MLN8054 orally on an empty stomach, with nothing by mouth two hours before and one hour after each dose except prescribed medications and water. Based on preclinical studies, 2,000 nmol/L was the estimated efficacious concentration (11), a dose of 0.21 mg/kg was projected to provide MLN8054 concentrations >2,000 nmol/L, and the terminal elimination half-life (t₁/₂) was projected to be approximately 27 hours, supporting once-daily (QD) dosing. The initial dosing regimen was 10 mg QD for 5 days/week on 2 consecutive weeks (days 1–5 and 8–12; QD-10D), with 14-day breaks in each cycle. The trial began with this intermittent administration schedule to reduce the risk of excessive bone marrow and/or mucosal toxicity. Additionally, preclinical studies of MLN8054 had shown antitumor activity with the intermittent administration schedule (5 days on/2 days off) in xenograft models. The plan was to increase the dose to QD dosing for 14 to 21 consecutive days after pharmacologically active exposure to MLN8054 was safely achieved and maintained with the QD-10D schedule. However, due to high levels of somnolence with the QD regimen in a concurrent phase I study (12, 13), particularly at higher doses, the protocol was amended to include divided 4-times-daily dosing for 14 consecutive days (QID-14D), with the highest dose at bedtime, to minimize daytime sedation and maximize exposure to potentially therapeutic concentrations of MLN8054 (14). Starting at the 45 mg dose level, oral methylphenidate 5 to 15 mg was also permitted during daytime dosing. The highest dose level and schedule that resulted in DLTs in 0 of 3 or in 0 to 1 of 6 patients was considered the MTD; additional patients, for a cumulative total of 9 to 12 patients, were to be enrolled to confirm this MTD.

AE information was collected throughout the study. Safety assessments were based on evaluation of AEs and serious AEs, 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.

Blood samples for pharmacokinetic analyses in the QD-10D cohorts were drawn once at baseline (day 1 predose), serially (predose and 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours postdose) for the first (day 1) and last (day 12) dose of cycle 1, and once daily on days 15 to 19. In the QID-14D cohorts, pharmacokinetic samples were obtained once at baseline and serially for the second dose on day 7 (predose and 0.5, 1.0, 1.5, 2, 3, 4, and 6 hours postdose). Plasma was extracted from blood immediately and stored at −70°C until analysis. Analysis of plasma samples used a validated liquid chromatography/tandem mass spectrometry assay. The lower and upper limits of quantification for the undiluted MLN8054 samples were 5 ng/mL and 2,500 ng/mL, respectively. Calculation of pharmacokinetic parameters was done using WinNonlin Professional Version 5.2. The following pharmacokinetic parameters were calculated: maximum plasma concentration (Cmax), time to maximum plasma concentration (Tmax), t₁/₂, trough plasma concentration (Cmin), area under the plasma concentration time curve over the dosing interval (AUC₀–₂₄ h for QD dosing and AUC₀–₆ h for QID dosing), and peak-to-trough ratio (Cmax/Cmin).

For patients in the QD cohorts, serial 3-mm skin punch biopsies for pharmacodynamic analyses were obtained at baseline and again 6 and 24 hours postdose on days 1 and 12 in cycle 1. For patients in the QID-14D cohorts, skin punch biopsies were obtained at baseline and on day 7 before and 2 to 4 hours after the second daily dose of MLN8054. Tumor biopsies also were obtained in the QD cohorts (baseline plus 6 and 24 hours postdose on day 1) and QID-14D cohorts (baseline and 2 to 4 hours after the second daily dose on day 7). The purpose of these biopsies was to detect inhibition of Aurora A kinase as measured by accumulation of mitotic cells. Assays implemented to monitor Aurora A kinase inhibition included increases in the mitotic index (skin and tumor) as well as decreases in aligned chromosomes and bipolar spindles of mitotic cells (tumor). Immunofluorescence analysis was done on skin and tumor sections, using two mitotic markers, pSer10 Histone H3 (pHistH3; Upstate Cell Signaling Solutions) and MPM2 (Upstate Cell Signaling Solutions) and MPM2 (Upstate Cell Signaling Solutions). Sections were mounted with 4’,6-diamidino-2-phenylindole Vectashield Hard Set Mounting Medium (Vector Laboratories). The mitotic index was determined by manual count of the number of mitotic cells per millimeter in four separate sections, normalized to the length of the basal epithelial layer. The mitotic index in tumor biopsies was evaluated by calculating the percentage of total cells (nuclei count) that were mitotic (pHistH3 immunopositive) within the proliferative tumor regions (Ki67 immunopositive) using automated image analysis. To determine the percentage of mitotic cells with aligned chromosome and bipolar spindles, tumor sections underwent immunofluorescence staining for α-tubulin and DNA. The stained sections were imaged by multiplane acquisition and the resultant Z-stack images were deconvolved and used to generate three-dimensional reconstructions of mitotic cells. These three-dimensional images were presented to four to six scorers in a random, blinded manner for scoring chromosome alignment and spindle bipolarity, and the majority call was used for the final determination.

Before receiving the first dose of MLN8054, patients underwent disease evaluation including physical examination, computed tomography and/or magnetic resonance imaging, and tumor markers, such as prostate-specific...
antigen, cancer antigen (CA)-125, and CA19-9, when applicable. Evaluations were repeated after every two cycles of MLN8054, and disease status was categorized using standard Response Evaluation Criteria in Solid Tumors (RECIST) v1.0 guidelines (15). Patients who had stable disease or a partial response continued therapy either until there was evidence of disease progression or until unacceptable treatment-related toxicity. Patients achieving a complete response to MLN8054 treatment continued therapy for at least two cycles beyond confirmation of the complete response; continued therapy until progressive disease or unacceptable treatment-related toxicity was permitted. Patients who tolerated the first cycle of therapy with MLN8054 could increase the dose of MLN8054 therapy in subsequent cycles of treatment after clinical experience established the safety of that increased dose level.

Expected enrollment was approximately 60 patients. The safety population included all patients who received at least one dose of study drug; the DLT population included all patients who received the study drug at the assigned dose level and had sufficient follow-up to determine if a DLT occurred. Descriptive statistics are reported for baseline values, safety, pharmacokinetics, and pharmacodynamics.

Results

Patient disposition and demographics

The 9 treatment cohorts included a total of 43 patients who were treated and were evaluable for safety (Table 1). Thirty-nine patients (91%) were evaluable for DLT. Forty-one patients (95%) were evaluable for pharmacokinetics.

Baseline demographic and disease characteristics for the safety population are summarized in Table 2. Approximately half of the patients were female (51%), and all (100%) were Caucasian. Median age was 60 years (range, 37–76 years). All patients had an ECOG performance status of 0 (72%) or 1 (28%). The most common tumor type was colorectal cancer (51%); a variety of other solid tumors were seen in <10% of patients each. Most patients had been heavily pretreated, with 65% having received ≥3 prior regimens.

The reasons for discontinuation were progressive disease (77%), patient’s withdrawal of consent (12%), AE (5%), unsatisfactory therapeutic response (5%), and symptomatic deterioration (2%). The median number of MLN8054 treatment cycles was 1 (range, 1–10). At least 95% of planned doses were administered in each cohort.

Dose-limiting toxicity

DLT is summarized in Table 1. All DLTs were transient, but a few patients discontinued treatment in the first cycle due to these DLTs. The only DLT seen with QD dosing was transaminitis in one of six patients in the QD-10D 20 mg cohort. Somnolence was seen in one patient each in the QID-14D 25-mg and 45-mg cohorts, and in none of the patients in the QID-14D 35-mg and 55-mg cohorts. The patient with a DLT of somnolence in the QID-14D 45-mg cohort had the dose of MLN8054 reduced and received the oral psychostimulant methylphenidate with the remaining treatment cycles. An oral methylphenidate dose of 5 mg, administered with each daytime dose of MLN8054, was generally sufficient to manage daytime somnolence. Escalation to QID-14D 60 mg and 70 mg did not result in any DLT of somnolence, but one of six patients in the QID-14D 70-mg cohort had a DLT of grade 3 hepatotoxicity. This patient had elevated liver function tests at baseline (AST = 70 U/L, ALT = 82 U/L, and bilirubin = 11.63 μmol/L) that increased by day 14 of cycle 1 (AST = 106 U/L, ALT = 200 U/L, and bilirubin = 12.14 μmol/L) and then returned to below baseline levels by the end of cycle 1. The dose was reduced to QID-14D 60 mg in cycles 2 and 3, during which liver function tests followed a similar pattern of increasing by day 14 of each cycle and then returning to below baseline during the treatment breaks. The dose of QID-14D 80 mg was above the MTD because DLT was seen in two of six patients (somnolence and transaminitis). A total daily dose of 70 mg, administered QID for 14 days of each 28-day cycle, was the estimated MTD, although this was not further evaluated in an expansion cohort.

Adverse events

Forty-one patients (95%) had at least one AE, and 39 (91%) had at least one drug-related AE (Table 3). The most common drug-related AEs were somnolence (79%) and asthenia (30%). Many patients received at least one other medication that could have contributed to somnolence; 13 patients (30%) received an opioid analgesic, 11 (26%)
received a benzodiazepine or other anxiolytic, and 4 (9%) received a hypnotic or sedative agent. Symptoms consistent with abuse or withdrawal were not reported.

Seven patients (16%) had at least one grade 3 or 4 drug-related AE; there were no fatal (grade 5) drug-related AEs. Grade 3 or 4 drug-related AEs included somnolence and transaminis in three patients (7%) each, and asthenia and hepatotoxicity in one patient (2%) each (Table 3). All of these events were transient and resolved after the dose of MLN8054 was reduced, held, or discontinued. One event of grade 3 transaminis and the event of grade 3 asthenia occurred after the first treatment cycle and therefore were not considered DLTs. The patient with transaminis elevation in cycle 3 had a previous DLT of hepatotoxicity in one patient (2%) each (Table 3). All of these events were transient and resolved after the dose of MLN8054 was reduced, held, or discontinued.

Eight patients (19%) had at least one serious AE. Of these, three patients (7%) had drug-related serious AEs, including one patient with somnolence in the 25-mg cohort, one patient with asthenia in the 70-mg cohort, and one patient with transaminis elevation in the 80-mg cohort. The serious AE of somnolence required hospitalization and discontinuation of study treatment in the first cycle, and thus was a DLT. This patient had been taking morphine for pain for one month prior to the event, which could have also contributed to somnolence. Two patients (5%) died on study; both deaths were due to disease progression and neither death was considered drug-related. One of these patients had advanced ovarian cancer with metastasis first diagnosed two years prior to entering the study; she received MLN8054 for 9 days in the first cycle before discontinuing treatment due to disease progression and died 16 days later. The other patient had advanced colorectal cancer with metastasis first diagnosed four months prior to enrollment in the study; this patient completed the first cycle of treatment and died of disease progression 11 days later. No clinically notable trends were observed for any other safety parameters (vital signs, hematology, physical examination, or electrocardiogram).

**Pharmacokinetics**

Mean MLN8054 plasma concentration-time profiles for QD-10D 10 and 20 mg are shown in Fig. 2. Table 4 summarizes pharmacokinetic parameters by treatment cohort. MLN8054 was absorbed over a short period of time, with an average time to \( T_{\text{max}} \) ranging from 1 to 2 hours postdose, and \( t_{1/2} \) was approximately 30 to 40 hours. Drug exposure was roughly dose proportional with QD dosing, and peak-to-trough ratios \( (C_{\text{max}}/C_{24 \text{ hr}}) \) after the final dose of the first cycle in the 10-mg and 20-mg cohorts were 4 and 7, respectively. None of the patients in the QD-10D cohorts had a trough concentration \( >2,000 \text{ nmol/L} \), the plasma concentration in which efficacy saturated in preclinical experiments (11). Pharmacokinetic analyses of the QID-14D cohorts showed that the geometric mean of peak-to-trough ratio \( (C_{\text{max}}/C_{6 \text{ hr}}) \) was between 1.3 and 1.7 over these seven dose cohorts, which was substantially lower than the ratio for single daily dosing regimens. In the 70-mg and 80-mg cohorts, the average MLN8054 plasma levels exceeded 2,000 nmol/L throughout the dosing period and were sustained to the end of treatment on day 14. In contrast, MLN8054 steady-state plasma concentrations were below 2,000 nmol/L in the 60-mg cohort and ranged from 1,000 to 2,000 nmol/L after treatment with the lower dose levels.

### Table 3. Drug-related adverse events: any grade in \( \geq 10\% \) of patients or grade \( \geq 3 \) in any patient

<table>
<thead>
<tr>
<th>Any adverse event</th>
<th>QD dosing</th>
<th>QID dosing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 43)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Any adverse event</td>
<td>39 (91)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>Somnolence</td>
<td>34 (79)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>Asthenia</td>
<td>13 (30)</td>
<td>1 (33)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>7 (16)</td>
<td>0</td>
</tr>
<tr>
<td>Anorexia</td>
<td>6 (14)</td>
<td>0</td>
</tr>
<tr>
<td>Transaminitis</td>
<td>3 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Any grade ( \geq 3 ) event*</td>
<td>7 (16)</td>
<td>0</td>
</tr>
<tr>
<td>Somnolence</td>
<td>3 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Asthenia</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Transaminitis</td>
<td>3 (7)</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE:** Values in parentheses are percentages.

**Abbreviations:** QD, once-daily dosing; QID, 4 times a daily dosing.

*No patient had a grade 5 drug-related adverse event.
Pharmacodynamics

An increase in skin mitotic index (number of pHistH3 immunopositive mitotic cells per millimeter of basal epithelial layer) was consistent with Aurora A kinase inhibition in several patients, most markedly at the highest dose cohorts (70 and 80 mg). A comprehensive evaluation of the pharmacodynamic effects of MLN8054 has been described elsewhere (16). Illustrative data from a patient in the QID-14D 80 mg dose cohort are shown in Fig. 3A. The average mitotic index from four individual skin sections from this patient increased from 0.26 prior to dosing to 5.00 and 2.96 on day 7, 2 to 4 hours before and 2 to 4 hours after the second daily dose, respectively. The difference in the mitotic index between the two day 7 biopsies was likely due to intrapatient sampling variation rather than a time-dependent or exposure/response effect. This patient also showed a 31% increase in the tumor mitotic index within proliferative regions (the percentage of pHistH3 immunopositive cells residing in Ki67 immunopositive regions; Fig. 3B). There was, however, no clear evidence for a decrease in the total Ki67 immunopositive area at the time of this tumor biopsy.

Table 4. Summary of pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Cohort</th>
<th>No. of patients</th>
<th>No. with pharmacokinetic data</th>
<th>Mean (SD)</th>
<th>Geometric mean peak-to-trough ratio (Cmax/Cmin)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>T_{max}, h</td>
<td>C_{max}, nmol/L</td>
</tr>
<tr>
<td>QD-10D (day 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg</td>
<td>3</td>
<td>3</td>
<td>1.2 (0.3)</td>
<td>923 (70)</td>
</tr>
<tr>
<td>20 mg</td>
<td>6</td>
<td>6</td>
<td>1.5 (0.9)</td>
<td>1,779 (440)</td>
</tr>
<tr>
<td>QID-14D (day 7, dose 2)</td>
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<td></td>
<td></td>
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<tr>
<td>25 mg</td>
<td>7</td>
<td>7</td>
<td>1.2 (0.3)</td>
<td>1,252 (669)</td>
</tr>
<tr>
<td>35 mg</td>
<td>3</td>
<td>3</td>
<td>1.0 (0.5)</td>
<td>1,169 (741)</td>
</tr>
<tr>
<td>45 mg</td>
<td>6</td>
<td>6</td>
<td>1.4 (1.2)</td>
<td>1,806 (604)</td>
</tr>
<tr>
<td>55 mg</td>
<td>3</td>
<td>3</td>
<td>0.8 (0.8)</td>
<td>2,173 (536)</td>
</tr>
<tr>
<td>60 mg</td>
<td>3</td>
<td>3</td>
<td>0.3 (0.6)</td>
<td>1,736 (185)</td>
</tr>
<tr>
<td>70 mg</td>
<td>6</td>
<td>6</td>
<td>2.3 (1.9)</td>
<td>3,498 (1,186)</td>
</tr>
<tr>
<td>80 mg</td>
<td>6</td>
<td>6</td>
<td>2.1 (1.8)</td>
<td>4,199 (2,804)</td>
</tr>
</tbody>
</table>

NOTE: C_{min}, minimum plasma concentration (trough value); AUC_{0-τ}, area under the concentration time curve from time zero to τ, where τ = 24 in the QD cohorts and τ = 6 in the QID cohorts.

*C_{min} was measured 24 hours after the daily dose on day 12 in the QD cohorts and 6 hours after the second dose on day 7 in the QID cohorts.

†Administered 5 consecutive days/week for 2 weeks (i.e., days 1–5 and 8–12).
To further corroborate that MLN8054 inhibited Aurora A kinase, chromosome alignment and spindle bipolarity were evaluated in mitotic cells from the tumor biopsies. Examples of mitotic cells stained for α-tubulin and DNA from predose and postdose biopsies are shown in Fig. 3C. In the patient from the 80-mg-dose cohort described above, there was a marked decrease in the percentage of mitotic cells with aligned chromosomes and bipolar spindles after 7 days of dosing with MLN8054, findings consistent with Aurora A kinase inhibition.

**Clinical responses**

No RECIST complete or partial responses were seen. Eighteen patients received only one cycle of MLN8054 treatment before discontinuing due to progressive disease or insufficient therapeutic effect. Three patients had four cycles or more with stable disease. One patient in the QID-14D cohort with non–small cell lung cancer and progressive disease with the most recent course of treatment (taxane monotherapy) had stable disease for four cycles (121 days) of MLN8054 treatment. One patient in the QID-14D 55-mg cohort with melanoma and no response better than stable disease during eight courses of treatment in the previous five years had stable disease for nine cycles (251 days) of MLN8054 treatment. One patient in the QID-14D 70-mg cohort with colorectal cancer and no response better than stable disease during seven courses of treatment in the previous three years had stable disease for four cycles (140 days) of MLN8054 treatment. One patient in the QID-14D 70-mg cohort with colorectal cancer and no response better than stable disease during seven courses of treatment in the previous three years had stable disease for seven cycles (210 days) of MLN8054 treatment. One patient in the QID-14D 70-mg cohort with colorectal cancer and no response better than stable disease during seven courses of treatment in the previous three years had stable disease for seven cycles (210 days) of MLN8054 treatment.
disease for five cycles (112 days) of MLN8054 treatment. All three patients eventually discontinued the study due to progressive disease or symptomatic deterioration. Two of these patients did not have notable changes in pharmacodynamic parameters, but the patient with colorectal cancer in the QID-14D 70-mg cohort had profound changes in mitotic index, aligned chromosomes, and bipolar spindles (Fig. 3B), consistent with Aurora A inhibition. This patient had MLN8054 concentrations >2,000 nmol/L from 1.5 to 4 hours after the second dose on day 7 and the trough concentration (C_{th}) of 1,960 nmol/L was slightly below 2,000 nmol/L. The other two patients with stable disease in the lower dose cohorts did not have any MLN8054 concentrations >2,000 nmol/L. Notably, grade 2 mucositis and reduced neutrophil counts, toxicities consistent with the desired mechanism to target dividing cells, were observed only in the highest cohort of QID-14D 80 mg, which was above the MTD.

Discussion

This phase I study of the safety, pharmacokinetics, and pharmacodynamics of MLN8054, the first-generation Aurora A kinase inhibitor, showed that dosing for 10 to 14 days of a 28-day cycle was feasible in patients with advanced tumors. To enable escalation of total daily doses, the schedule was modified to four divided doses daily (QID-14D) to mitigate benzodiazepine-like effects that seemed to be associated with peak plasma concentrations. Similarly, the addition of a low dose of the oral psychostimulant methylphenidate (5–15 mg; administered with each daytime dose of MLN8054) provided some utility to manage somnolence and allowed for extended dosing. MLN8054 is structurally related to benzodiazepines and was shown in preclinical studies to bind the GABA_A receptor, which may contribute to reversible benzodiazepine-like effects in humans. Despite divided daily dosing and the addition of methylphenidate with daytime doses, transient somnolence and transaminitis/hepatotoxicity prevented escalation to doses that showed more robust bioactivity or were associated with clinical antitumor effects. Using a QID schedule with 15 mg administered three times during the day and 25 mg at night, the total daily dose of 70 mg was generally tolerable with one DLT in six patients. Thus, a total daily dose of 70 mg, administered as divided doses (QID) for 14 days of each 28-day cycle, is the estimated MTD. Because of the lack of evidence for mechanistic bioactivity or antitumor effects at the estimated MTD, a dosing regimen for future phase II development (the recommended phase II dose) could not be determined. Nonetheless, these methods and results, particularly the pharmacokinetic sampling and biomarker methodologies, were informative for ongoing clinical development of agents to target Aurora A kinase. Future clinical development of Aurora A kinase inhibitors needs to consider relationships between peak plasma levels and tolerability, single versus divided doses, and the balance between tolerability and the ability to achieve sustained exposures associated with desired mechanisms.

Pharmacokinetic analyses showed that MLN8054 was absorbed rapidly (T_{max} 1–2 hours), t_{1/2} was 30 to 40 hours, and drug exposure was roughly dose proportional with QD dosing. Dividing the daily dose into QID doses resulted in substantially lower peak-to-trough ratios and MLN8054 concentrations above the target of 2,000 nmol/L in the highest dose cohorts of QID-14D 70 mg and 80 mg. Four of six patients in the QID-14D 70-mg cohort and one of six patients in the QID-14D 80-mg cohort were dosed beyond one cycle. One of the patients in the QID-14D 70-mg cohort had a trough MLN8054 concentration near 2,000 nmol/L in the first cycle and this patient showed profound changes in pharmacodynamic parameters and stable disease for five cycles. Two other patients from lower dose cohorts had stable disease for four and nine cycles, respectively, but these patients did not have MLN8054 concentrations >2,000 nmol/L or notable changes in pharmacodynamic parameters.

The onset of somnolence one to two hours after dosing corresponded to the T_{max} in several patients during the early days of dosing prior to achieving steady-state exposures, suggesting that somnolence was a C_{max}-related toxicity and supporting the design to reduce peak-to-trough ratio by changing the schedule to QID doses. At intermediate dose levels, the QID-14D regimens did lead to reduced peak-to-trough levels and were associated with reduced frequency of somnolence. Although all patients (6 of 6) reported somnolence in the QD-10D 20-mg cohort, somnolence was reported by only a minority of patients given doses >20 mg with the QID schedule, up to total doses of 45 to 55 mg/day. Somnolence emerged as a more frequent toxicity at the higher total daily dose levels even with the QID regimen. There were no complete or partial responses, and many patients received only one cycle of MLN8054 treatment before discontinuing due to progressive disease or unsatisfactory therapeutic effect. Evidence of antiproliferative effects including grade 2 oral mucositis and reduced neutrophil counts were observed only in the highest cohort of 80 mg daily, a dose considered to be above the MTD. Skin biopsy findings provided evidence of Aurora A kinase inhibition.

A distinctive aspect of this study was the inclusion of tumor biopsies pretreatment and posttreatment, which revealed evidence of Aurora A kinase inhibition within tumor tissue. Increases in the mitotic index in the skin and tumor biopsies and decreases in aligned chromosomes and bipolar spindles in one patient in the 80-mg cohort were consistent with Aurora A inhibition. Although there was evidence of Aurora A kinase inhibition with MLN8054 in this patient, which was also seen in patients in another study (16), the most prominent pharmacodynamic activity was observed only at doses where somnolence or transaminitis were dose limiting. Interestingly, pHistH3 is an Aurora B kinase substrate and would be expected to diminish if Aurora B kinase were inhibited. Therefore, the increased mitotic index in skin
and tumor biopsies is consistent with selective inhibition of Aurora A kinase relative to Aurora B kinase by MLN8054 in humans. To our knowledge, this is the first time an increase in the mitotic index and decreases in aligned chromosomes and bipolar spindles, consistent with Aurora kinase A inhibition, have been shown in human tumor specimens. Collectively, the pharmacodynamic results of these studies suggest that although MLN8054 had the intended effects at the pharmacodynamic level, DLTs may have prevented escalation of MLN8054 to a clinically effective dose.

In conclusion, Aurora A kinase was inhibited in the skin and tumors of patients in this phase I study. However, dose-limiting somnolence and transaminitis occurred before clinical antiproliferative effects or objective tumor responses. A more potent, second-generation Aurora A kinase inhibitor, MLN8237, was developed by modifying the structure of MLN8054 to reduce binding to the GABA_A receptor (17, 18). MLN8237 is being investigated in dose-finding phase I studies (19, 20) to determine if these modifications will permit escalation to a dose that is both effective and well tolerated.

Disclosure of Potential Conflicts of Interest

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References

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