Phase I Pharmacokinetic/Pharmacodynamic Study of MLN8237, an Investigational, Oral, Selective Aurora A Kinase Inhibitor, in Patients with Advanced Solid Tumors

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Abstract

Purpose: Aurora A kinase (AAK) is a key regulator of mitosis and a target for anticancer drug development. This phase I study investigated the safety, pharmacokinetics, and pharmacodynamics of MLN8237 (alisertib), an investigational, oral, selective AAK inhibitor, in 59 adults with advanced solid tumors.

Experimental Design: Patients received MLN8237 once daily or twice daily for 7, 14, or 21 consecutive days, followed by 14 days recovery, in 21-, 28-, or 35-day cycles. Dose-limiting toxicities (DLT) and the maximum-tolerated dose (MTD) for the 7- and 21-day schedules were determined. Pharmacokinetic parameters were derived from plasma concentration-time profiles. AAK inhibition in skin and tumor biopsies was evaluated and antitumor activity assessed.

Results: Neutropenia and stomatitis were the most common DLTs. The MTD for the 7- and 21-day schedules was 50 mg twice daily and 50 mg once daily, respectively. MLN8237 absorption was fast (median time to maximum concentration, 2 hours). Mean terminal half-life was approximately 19 hours. At steady state, pharmacodynamic effects were shown by accumulation of mitotic and apoptotic cells in skin, and exposure-related increases in numbers of mitotic cells with characteristic spindle and chromosomal abnormalities in tumor specimens, supporting AAK inhibition by MLN8237. Stable disease was observed and was durable with repeat treatment cycles, administered over 6 months, in 6 patients, without notable cumulative toxicity.

Conclusions: The recommended phase II dose of MLN8237 is 50 mg twice daily on the 7-day schedule, which is being evaluated further in a variety of malignancies, including in a phase III trial in peripheral T-cell lymphoma. Clin Cancer Res; 18(17); 4764–74. ©2012 AACR.
Translational Relevance

The Aurora kinases, and Aurora A kinase (AAK) in particular, are enzymes that play a key role in regulating normal mitotic progression, and as such, are rational targets for cancer therapy. This phase I dose-escalation study determined the safety, dose-limiting toxicities, and maximum tolerated dose of MLN8237 (alisertib), an investigational, oral, selective, small-molecule inhibitor of AAK, in 59 adults with advanced solid tumors. Safety and pharmacokinetics were studied in different dosing schedules and a recommended phase II dose was established. Evidence for pharmacokinetic-pharmacodynamic relationships in skin and tumor tissue provides support for AAK inhibition by MLN8237. MLN8237 continues to be evaluated in phase I, II, and III clinical studies in a variety of solid tumors and hematologic malignancies.

perturbation results in mitotic spindle defects, mitotic delay, and cell death (10–15). Several lines of evidence support a link between increased AAK expression and tumorigenesis: AAK overexpression is associated with the aneuploidy and centrosome amplification commonly seen in tumor cells (16); AAK overexpression has been reported in a variety of solid tumors and hematologic malignancies (16–23); furthermore, AAK is oncogenic and can transform normal cells in experimental systems (16, 23). AAK is, therefore, a rational target for anticancer therapy.

As reviewed recently (24), an estimated 30 small-molecule inhibitors of Aurora kinases are currently undergoing preclinical and clinical evaluation (25–31). A number of these compounds are pan-Aurora kinase inhibitors that show inhibitory activity against both AAK and Aurora B kinase (ABK), as well as, in some cases, Aurora C kinase. However, evidence suggests that these pan-Aurora kinase inhibitors result in a phenotype reflective of ABK inhibition (1, 24). In addition, a number of inhibitors selective for AAK or ABK are under development. Such selective inhibition may result in a different therapeutic index and toxicity profile compared with pan-Aurora kinase inhibition, associated with the toxicities due to inhibition of either AAK or ABK, rather than both. The relative merits of selectively targeting AAK or ABK remain to be determined, although data suggest that both approaches offer antineoplastic activity (24, 29).

MLN8237 (alisertib) is an investigational, orally administered, small-molecule inhibitor that is selective for AAK (32, 33). MLN8237 was developed, in part, on the basis of preclinical (25) and clinical studies (34–36) with a first-generation agent, MLN8054. MLN8054 showed sustained growth inhibition in multiple human tumor xenografts, with phenotypes consistent with AAK inhibition, in preclinical studies (25). In clinical studies, similar pharmacodynamic effects were reported, indicating AAK inhibition in skin and tumor tissues (34–36); however, dose escalation was stopped, and the MLN8054 development program discontinued, based on benzodiazepine-like somnolence and neurocognitive changes (34). Consistent with the expected activity of an AAK inhibitor, treatment with the second-generation agent MLN8237 has been shown to be associated with an accumulation of cells with abnormal mitotic spindles, leading to decreased proliferation and apoptosis in a range of human tumor cell lines (37–43). MLN8237 has shown promising single-agent antitumor activity in animal xenograft models (32, 41, 44) and enhanced antitumor activity in combination with standard agents in hematologic and solid tumor models (39, 40, 42, 43, 45).

Here we report results from a phase I clinical trial of MLN8237 in patients with advanced solid tumors (NCT00651664). The safety, pharmacokinetics, pharmacodynamics, and antitumor activity of MLN8237 are described. As with MLN8054, MLN8237 is structurally related to the benzodiazepines. Like other members of this family, MLN8237 has affinity for the GABA_A,α1 receptor (35), but it was selected for clinical development because of greater potency for AAK inhibition relative to MLN8054; thus, central nervous system (CNS) effects, such as somnolence, confusion, dizziness, and gait disturbance, were of particular interest during safety monitoring. A dose for further clinical development in adults is recommended.

Materials and Methods

Patients

Patients 18 years or older of age with histologically or cytologically confirmed metastatic and/or advanced solid tumors (including lymphomas) for which there was no standard curative or life-prolonging treatment were eligible for enrollment. Patients were required to have an Eastern Cooperative Oncology Group performance status of 0 or 1, radiographically or clinically evaluable disease, and adequate hematologic, renal, and hepatic function, defined as follows: absolute neutrophil count 1,500 cells/mm^3 or more; platelet count 100,000/mm^3 or more; serum creatinine 1.6 mg/dL or less; creatinine clearance 40 mL/min or more; bilirubin 1.5 × upper limit of normal (ULN) or less; aspartate and alanine aminotransferases and alkaline phosphatase 2.5 × ULN or less. To meet the exposure-pharmacodynamic assessment goals of the study, it was required that most patients had accessible tumors for sampling of pre- and posttreatment biopsies.

Ineligibility criteria included malignancy with extensive bone marrow involvement, more than 4 prior cytotoxic regimens, known gastrointestinal disease or procedures that could interfere with the oral absorption or tolerance of MLN8237, prior peripheral blood stem cell or bone marrow transplantation, prior radiation therapy involving 25% or more of hematopoietically active bone marrow, presence of CNS metastases, a history of uncontrolled sleep apnea syndrome, or other conditions that could result in excessive daytime sleepiness.
All patients gave written informed consent, and ethics committees at the participating institutions and regulatory authorities approved the study, which followed the Declaration of Helsinki and Good Clinical Practice guidelines.

Study design

This open-label, phase I, dose-escalation study was conducted at 2 Spanish academic institutions. MLN8237 (structure published previously; refs. 32, 44), was administered orally on an empty stomach, with patients remaining nil-by-mouth, except for water and prescribed medications, for 2 hours before and 1 hour after each dose. Dose escalation proceeded via a standard 3 + 3 design based upon the occurrence of dose-limiting toxicities (DLT) during cycle 1 of treatment. The starting dose and schedule were selected on the basis of animal toxicity studies (Millennium Pharmaceuticals, Inc., data on file), which supported 7 days’ consecutive dosing followed by 14 days’ rest before a new treatment cycle. Patients were scheduled to receive a starting dose of MLN8237 5 mg once daily for 7 consecutive days followed by a 14-day rest period (21-day cycles), with dose doubling in successive cohorts, reduced to a maximal 40% escalation when 2 or more patients at a given dose experienced grade II MLN8237-related toxicities or 1 patient experienced a grade III or higher MLN8237-related toxicity. Dose escalation was stopped if DLTs occurred in 2 or more patients of 3 to 6 patients enrolled to any dose level. The maximum-tolerated dose (MTD) was designed to be the highest dose level in which DLTs occurred in less than or equal to 1 of 6 patients. Following preliminary safety and pharmacokinetic analyses with MLN8237 dosing in the once daily schedule, twice daily dosing was subsequently explored. Upon determining the MTD for twice daily dosing on the 7-day schedule, an expansion cohort was treated at this dose to better define safety, pharmacokinetic, and pharmacodynamic effects. Subsequent patients received MLN8237 for 14 and 21 consecutive days (in 28- and 35-day cycles, respectively; Supplementary Fig. S1). The investigation of longer schedules was based on nonclinical data, suggesting that longer exposure durations may improve antitumor activity (32). Patients received repeat cycles of MLN8237 treatment until disease progression or unacceptable toxicity.

Objectives

The primary objective was to determine DLTs and the MTD for MLN8237. Secondary objectives were to describe MLN8237 pharmacokinetics, to evaluate the pharmacodynamic effects of MLN8237, reflective of AAK inhibition in skin and tumor, and to describe antitumor activity.

Assessments

Safety. Adverse events were graded by National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0. DLTs were defined as any of the following MLN8237-related events during the first treatment cycle: grade IV neutropenia during dosing or lasting 7 or more days during the rest period, alone or associated with fever and/or infection; grade IV thrombocytopenia; grade III or higher nausea, emesis, and/or diarrhea despite maximal supportive care; other grade III or higher nonhematologic toxicities with the exceptions of grade III arthralgia or myalgia, brief (<1 week) grade III fatigue, or alopecia of any grade; other MLN8237-related toxicities that resulted in a delay in the start of the next treatment cycle by more than 1 week; or other grade II or higher nonhematologic toxicities that required MLN8237 dose reduction or discontinuation.

Pharmacokinetics. Serial blood samples were drawn before and at protocol-specified time points after MLN8237 dosing during the first treatment cycle. MLN8237 plasma concentrations were measured by validated liquid chromatography/tandem mass spectrometry for noncompartmental pharmacokinetics analysis. Detailed pharmacokinetics methodology is provided in the Supplementary Information.

Pharmacodynamics and AAK inhibition. The pharmacodynamics- evaluable population included patients who received at least one dose of MLN8237 and had either a baseline skin punch biopsy and one or more additional skin punch biopsies taken during treatment cycle 1, or a baseline tumor biopsy and at least one other tumor biopsy taken during cycle 1. The pharmacodynamic effects of MLN8237 on AAK inhibition were evaluated by quantifying posttreatment changes in the number of phosphohistone H3 (pHistH3)- and mitotic protein monoclonal (MPM2)-positive mitotic cells in the basal epithelium of the skin, the number of pHistH3-positive mitotic cells in Ki67-positive proliferative regions of tumor biopsies, the number of apoptotic cells in skin biopsies, and the incidence of mitotic cells with aligned chromosomes and bipolar spindles in tumor biopsies stained for α-tubulin and DNA. Skin biopsies were collected before dosing on day 1, 6 to 8 hours postdose on days 1, 7, 14, and 21, and 24 to 28 hours postdose on days 7 and 14. Tumor biopsies were collected before dosing on day 1 and 6 to 8 hours postdose on days 1, 7, 14, and 21. Immunolabeling of skin and tumor biopsies for the quantification of mitotic cells and of tumor biopsies for the assessment of chromosome alignment and spindle bipolarity were conducted as previously described (36). For detection of apoptotic cells, 5-μm sections of formalin-fixed, paraffin-embedded skin punch biopsies were deparaffinized and stained by standard methods with hematoxylin and eosin using a Leica Autostainer XL (Meyer Instruments, Inc.).

In patients with matched day 7 pharmacokinetic and tumor pharmacodynamic data, the relationship between MLN8237 steady-state exposure [area under the concentration–time curve (AUC0-7)] and tumor pharmacodynamics was evaluated graphically.

Assessment of clinical disease and response. Before receiving MLN8237, the baseline disease status of patients was assessed via standard clinical examinations. Response was assessed after every 2 cycles (or every 3 cycles for some
patients who remained on-study for more than 6 months) by RECIST (46).

Results

Patient characteristics

Fifty-nine patients received MLN8237. The baseline characteristics of patients are summarized in Table 1. The median age was 61 years (range 30–78), and a variety of solid tumor types were represented, with colorectal cancer being the most frequent (46%). All patients had received one or more prior therapies, and 63% had received 3 or more prior therapies. Patients received a median of 2 cycles (range 1–29) of MLN8237.

Safety

All 59 patients received at least one dose of MLN8237 and were included in the safety population. The first cohort (n = 3) received 5 mg once daily on the 7-day schedule. Thereafter, with knowledge that MLN8237 was well tolerated at higher once daily doses using the same treatment schedule in a concurrent phase I study by Dees and colleagues (47), subsequent groups received 80 mg (n = 3) or 150 mg (n = 3) once daily on the 7-day schedule.

Drug-related somnolence was associated with high once daily doses (Table 2). Four of 6 patients treated at the 80- and 150-mg once daily dose levels experienced grade II somnolence. On the basis of the apparent association between high once daily dosing and high C_{max} and the occurrence of somnolence in the phase I study of the first-generation agent MLN8054 (34), and per the change in dosing schedule used in this prior study, subsequent cohorts in this study received twice daily doses of MLN8237: 50 mg (n = 14), 100 mg (n = 6), 75 mg (n = 3), or 60 mg (n = 6) on the 7-day schedule. The 7-day twice daily schedule was associated with a reduced frequency, and severity of somnolence compared with similar total daily doses administered once daily. Only 3 of 14 patients treated at 50 mg twice daily reported somnolence, all having grade I severity. Two patients (1 grade II, 1 grade III) at 60 mg twice daily, 1 (grade II) at 75 mg twice daily, and 3 (all grade II) at 100 mg twice daily also reported somnolence. MLN8237-related somnolence was typically first seen in cycle 1 of treatment and resolved within 7 days of onset. No other CNS toxicities were reported in more than one patient overall.

No DLTs were observed during cycle 1 in the 3 once daily dose cohorts on the 7-day schedule. Similarly, no DLTs were observed in the first 3 patients enrolled to the 50-mg twice daily dose cohort. In the 100-mg twice daily dose cohort, 3 of 6 patients with colorectal cancer experienced DLTs of grade III stomatitis and grade IV neutropenia during the rest period on day 9 or 10 of cycle 1. In all 3 patients, the events of grade III stomatitis resolved in 14, 20, and 23 days, and the events of grade IV neutropenia resolved in 11, 12, and 17 days, respectively. One patient subsequently discontinued treatment due to the reoccurrence of grade II stomatitis in cycle 2. In the subsequent 75-mg twice daily dose cohort, 2 of 3 patients experienced DLTs: 1 patient with colorectal cancer reported grade II stomatitis on day 10 of cycle 1, which required MLN8237 dose reduction and resolved in 14 days; another patient with soft-tissue sarcoma reported grade IV neutropenia on day 21 of cycle 1, which also required dose reduction and resolved in 14 days. Similarly, in the 60-mg twice daily dose cohort, 2 of 6 patients experienced DLTs: 1 patient with colorectal cancer reported grade III somnolence on day 1 of cycle 1, which required MLN8237 dose reduction and hospitalization and resolved in 2 days; the other patient, who had renal cell carcinoma, reported grade IV neutropenia on day 10 of cycle 1, which also resulted in dose reduction and resolved in 25 days. Thus, this cohort was subsequently expanded to a total of 14 patients, with no DLTs reported during cycle 1 in the additional 11 patients and so this dose and schedule were determined to be the MTD and RP2D.

Per the protocol-specified study design (Supplementary Fig. S1), after evaluating results from the 7-day schedule, 14- and 21-day dosing schedules were subsequently explored in additional cohorts (14-day 50 mg once daily, n = 7; 21-day 50 mg once daily, n = 7; 21-day 70 mg once daily, n = 7). Only one patient (with head and neck cancer) enrolled to the 14-day 50 mg once daily cohort reported a DLT of grade IV thrombocytopenia, which occurred on day 24 of cycle 1, required dose reduction and supportive care, and resolved in 38 days. Similarly, only one patient (with soft-tissue sarcoma) enrolled to the 21-day 50 mg once daily cohort experienced a DLT of grade IV decreased platelet count, which occurred on day 19 of cycle 1, resulted in dosing being held and was not recorded as having resolved. In the 21-day 70 mg once daily dose cohort, 2 of 7 patients

Table 1. Patient demographics and baseline characteristics

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<thead>
<tr>
<th>Characteristic</th>
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<tr>
<td>Male, n (%)</td>
<td>37 (63)</td>
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<tr>
<td>Caucasian, n (%)</td>
<td>59 (100)</td>
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<tr>
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| Other tumor types were anal carcinoma (n = 2), bone sarcoma (n = 1), breast cancer (n = 1), cervical carcinoma (n = 1), esophageal cancer (n = 1), gastric cancer (n = 1), liver cancer (n = 1), non–small cell lung cancer (n = 1), pancreatic carcinoma (n = 1), small cell lung cancer (n = 1), uterine carcinoma (n = 1), and tumors of unknown origin (n = 3).

*Other* tumor types were anal carcinoma (n = 2), bone sarcoma (n = 1), breast cancer (n = 1), cervical carcinoma (n = 1), esophageal cancer (n = 1), gastric cancer (n = 1), liver cancer (n = 1), non–small cell lung cancer (n = 1), pancreatic carcinoma (n = 1), small cell lung cancer (n = 1), uterine carcinoma (n = 1), and tumors of unknown origin (n = 3).
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<th>Once daily 7-d 80 mg (n = 3)</th>
<th>Once daily 7-d 150 mg (n = 3)</th>
<th>Twice daily 7-d 50 mg (n = 14)</th>
<th>Twice daily 7-d 60 mg (n = 6)</th>
<th>Twice daily 7-d 75 mg (n = 3)</th>
<th>Twice daily 7-d 100 mg (n = 6)</th>
<th>Once daily 14-d 50 mg (n = 7)</th>
<th>Once daily 21-d 50 mg (n = 7)</th>
<th>Once daily 21-d 70 mg (n = 7)</th>
<th>Total (N = 59), n (%)</th>
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experienced DLTs; 1 patient with ovarian cancer reported grade II stomatitis and diarrhea on days 13 and 16 of cycle 1, which required dose reduction, supportive care, and hospitalization, and resolved in 15 and 5 days, respectively; the other patient, who had anal carcinoma, reported grade III enteritis, which also required dose reduction, supportive care, and hospitalization, and resolved in 13 days. The MTD on the 21-day schedule was thus determined to be 50 mg once daily. One of 7 patients treated at this dose level reported somnolence (grade II), whereas 5 of the 7 patients (3 grade I, 2 grade II) treated at 70 mg once daily on the 21-day schedule reported this adverse event.

All 59 patients reported at least 1 adverse event, and 46 (78%) patients had drug-related adverse events. Drug-related grade III or higher adverse events were reported in 25 (42%) patients (Table 2); of these, over half (n = 14) received total daily doses exceeding the recommended phase II dose (RP2D) of 50 mg twice daily on the 7-day schedule. Drug-related adverse events are summarized in Table 2. Grade III or greater neutropenia was observed in 20 patients treated at various dose levels, but typically resolved in less than 7 days without G-CSF support; grade III or higher febrile neutropenia was infrequent (2 patients, 3%). Peripheral neuropathy, a toxicity associated with microtubule-perturbing antimitotic agents, was not a significant toxicity associated with MLN8237 treatment in this study.

Serious adverse events were reported in 26 (44%) patients across multiple dose levels, and those related to MLN8237 included diarrhea (5 patients, 8%), stomatitis (4 patients, 7%), and neutropenia (4 patients, 7%). Progressive disease was the main reason cited for discontinuation of MLN8237 treatment (45 of 59 patients, 76%). Two (3%) patients discontinued treatment due to adverse events; one patient at the 7-day 100 mg twice daily dose level discontinued due to grade II stomatitis, as noted earlier, and 1 patient at the 21-day 50 mg once daily dose level discontinued due to a grade III femur fracture. There were 2 on-study deaths due to progressive colorectal cancer, reported in patients treated at the 75 mg twice daily 7-day and 50 mg once daily 14-day dose levels.

Pharmacokinetics

All 59 patients were included in the pharmacokinetics—evaluable population. Figure 1 shows mean plasma concentration–time profiles for MLN8237 twice daily dosing on the 7-day schedule. Pharmacokinetic parameters for once daily dosing in the 7-, 14-, and 21-day schedules and twice daily dosing on the 7-day schedule are summarized in Supplementary Tables S1–S4. MLN8237 absorption was fast, with a median time of first observed maximum concentration (Tmax) of 2 hours (range 1–5). Examination of steady-state exposures across all dose levels revealed dose-dependent increases in steady-state area under the plasma concentration–time curve from time zero to 24 hours (AUC0–24 h) and maximum serum concentration (Cmax). At the RP2D of 50 mg twice daily on the 7-day schedule, the geometric mean steady-state average concentration was approximately 1.5 μmol/L [coefficient of variation (CV) 44%, n = 9], which exceeded the estimated steady-state plasma concentration of approximately 1 μmol/L associated with saturating pharmacodynamic effects and antitumor activity in preclinical models (48). The overall mean steady-state terminal half-life was 19.2 hours (CV 50%, n = 43). Consistently, steady-state MLN8237 exposures were achieved by 1 week after treatment initiation based on examination of AUC0–t on days 7/14–21 from cohorts receiving the 14-day and 21-day dosing schedules.

The overall mean peak/trough ratio for once daily dosing was 4.9, with a mean accumulation ratio of 1.9. The overall mean peak/trough ratio for twice daily dosing was 2.7, with a mean accumulation ratio of approximately 3.0.

Pharmacodynamics

Fifty-eight (98%) patients were included in the pharmacodynamics—evaluable population.

**SKIN pharmacodynamic effects.** At higher dose levels (including the RP2D of 50 mg twice daily on the 7-day schedule), pharmacodynamic effects in the skin were observed 6 hours after the first MLN8237 dose on day 7, which was reflected in increases compared with baseline in both the MI and apoptotic index (AI) within the basal epithelium (Fig. 2B and D). Among all patients, the mean...
MI at 6 hours post first dose on day 7 was 3.633, which ranged from 0.193 for the 5-mg once daily 7-day dose cohort to 9.150 for the 100-mg twice daily 7-day dose cohort, and was 3.489 for the 50-mg twice daily 7-day 2D dose cohort. Similarly, the mean AI at this time point was 1.842, which ranged from 0.040 for the 5-mg once daily 7-day dose cohort to 6.040 for the 75-mg twice daily 7-day 2D dose cohort. This effect was maintained for 24 hours after the first day 7 MLN8237 dose (Supplementary Fig. S2A) but was not seen on day 1 of MLN8237 dosing (Fig. 2A; overall mean MI: 0.389; and Fig. 2C; overall mean AI: 0.071). On the 14-day and 21-day once daily schedules, which were typically associated with lower total daily doses compared with the 7-day schedule, changes from baseline in MI and AI were less pronounced in skin biopsies taken 6 and 24 hours after the final MLN8237 dose (Supplementary Figs. S2 and S3; overall mean MI: 0.260–0.570; overall mean AI: 0.105–0.130 across these time points).

**Tumor pharmacodynamic effects.** Pharmacodynamic effects were also observed after MLN8237 administration in evaluable patients with paired tumor biopsies. Some tumors displayed increases in MI after dosing, whereas others displayed decreases (Supplementary Fig. S4). The variability in tumor MI may be consistent with dual mechanisms of mitotic arrest and mitotic slippage, observed after treatment with AAK inhibitors (41). Many tumors also displayed a marked decrease in the percentage of mitotic cells with aligned chromosomes and bipolar spindles after dosing (Fig. 3A and B; Supplementary Fig. S5). Observation of pharmacodynamic effects in tumors from patients treated at the RP2D of 50 mg twice daily on the 7-day schedule confirmed biologically active exposure levels within tumors.

Matched day 7 AUC(0-τ) data and tumor pharmacodynamics data were available for 9 patients and permitted assessment of potential relationships between steady-state MLN8237 exposure and pharmacodynamic effects reflecting AAK inhibition in tumor tissue. An exposure-related decrease in chromosome alignment and spindle bipolarity in mitotic tumor cells was observed, consistent with AAK inhibition by MLN8237 (Fig. 3C and D).
Antitumor activity
Twenty-two (37%) patients achieved stable disease as a best response; the median duration of stable disease was 7.3 months (range 0.8–22.8; lower value represents a censored observation). Stable disease was durable for more than 6 months in 6 patients and for approximately 1 year or longer in 4 patients with diagnoses of colorectal cancer, chondrosarcoma, leiomyosarcoma, and liposarcoma. Antitumor activity was observed in one patient with a metastatic, treatment-resistant, retroperitoneal pleomorphic liposarcoma that showed a maximum 25.7% reduction in diameter from baseline as measured by serial computed tomography (CT) scans (Fig. 4). This patient was initially enrolled to the 100-mg twice daily dose level (7-day schedule), but underwent dose reduction to 75 mg twice daily, and then to 50 mg twice daily because of febrile neutropenia with diarrhea and remained on treatment for 21 cycles over 1.2 years.

Discussion
This phase I dose–escalation study examined the safety, DLTs, and MTD of MLN8237, an oral, selective inhibitor of AAK, in adults with advanced malignancies. The study was also designed to analyze pharmacokinetic and pharmacodynamic relationships using serial biopsies of both normal (skin) and tumor tissue. Collectively, these informed the selection of a RP2D of 50 mg twice daily on the 7-day schedule. Specifically, this was the MTD on the 7-day schedule, whereas dose escalation did not proceed beyond 50 mg once daily with extended MLN8237 dosing; thus, the 7-day schedule enabled maximal daily dosing of MLN8237.

Figure 3. Changes in the percentage of mitotic cells with aligned chromosomes and bipolar spindles in patients with evaluable predose and day 7, 6-hour postdose tumor biopsies by total daily exposure (A) and (B), or relative exposure (AUC_{0–24} h) and (C).
This was important with regard to achieving a geometric mean steady-state plasma concentration in excess of that associated with saturating pharmacodynamic effects and antitumor activity in preclinical models; furthermore, twice daily dosing was associated with lower peak plasma levels and a reduced mean peak/trough ratio than once daily dosing. Associated with the pharmacokinetic findings, the daily dose of MLN8237 on the 50 mg twice daily 7-day schedule resulted in more robust pharmacodynamic effects in skin and tumor tissue, indicative of biologically active exposure levels, than the lower once daily doses used on the 14-day and 21-day schedules.

Our safety findings showed that the most common DLTs observed with MLN8237 were neutropenia and stomatitis, which were particularly evident among patients who received a total daily dose in excess of 100 mg. These toxicities reflect the pharmacologic activity of MLN8237 in inhibiting AAK activity in highly proliferative tissues and are consistent with the toxicities observed in phase I studies of the first-generation AAK inhibitor MLN8054 (34, 35) and other Aurora kinase inhibitors (1, 24). Furthermore, they were typically readily reversible, with grade III or higher neutropenia resolving within a week, generally without G-CSF support. As a laboratory finding without overt clinical significance, grade III neutropenia was thus not included within the definition of DLT in this study because of the known effects of AAK inhibition; however, it would have been recorded as a DLT if it led to treatment delay of more than 1 week due to failure of hematologic recovery from the previous cycle of treatment. Further consideration of this toxicity and recommended dosing may be required when combining an AAK inhibitor with other chemotherapeutic agents associated with hematologic toxicity.

In addition to neutropenia and stomatitis, other common MLN8237-associated toxicities included alopecia, leukopenia, and somnolence. The CNS effect of somnolence is consistent with the benzodiazepine-like structure of MLN8237 and was particularly evident in patients receiving individual doses in excess of 50 mg, for example, being observed in 4 of 6 patients treated with MLN8237 80 or 150 mg once daily on the 7-day schedule; however, the frequency and severity of these events were reduced with twice daily dosing of MLN8237 at lower individual doses, which was designed to reduce peak plasma levels while maintaining overall systemic exposures. At the RP2D of 50 mg twice daily on the 7-day schedule, toxicities were generally manageable with supportive care and, with the exception of alopecia, were reversible during the rest period between cycles.

Determination of the clinical pharmacokinetic properties of MLN8237 revealed a dose-dependent increase in steady-state exposure over a 5- to 200 mg/d dose range, supporting the use of twice daily dosing to increase MLN8237 steady-state exposure compared with once daily dosing. MLN8237 absorption was fast with an overall median \( T_{\text{max}} \) of 2 hours, and steady-state concentrations were achieved within 1 week of dosing. At the RP2D, the geometric mean steady-state average concentration exceeded the plasma concentration associated with saturating levels of pharmacodynamic effects and efficacy in mouse xenograft studies (1 \( \mu \text{mol/L} \); ref. 48). This finding was reflected in the robust pharmacodynamic effects seen at the RP2D and higher doses.

The exposure–effect relationship between steady-state MLN8237 plasma levels and the magnitude of AAK inhibition indicate pharmacologic effects in both normal (skin) and tumor tissue, and provide support for the proposed dominant mechanism of action of the drug to modulate AAK. Skin and tumor biopsies taken on day 1 of treatment showed minimal change in the frequency of mitotic/apoptotic cells; however, after 7 days of treatment, robust and consistent pharmacodynamic effects were observed. The variable outcomes in tumor MI are consistent with the dual mechanisms of mitotic arrest and mitotic slippage described in tumors treated with antimitotic agents, including AAK inhibitors (36). Importantly, exposure-related decreases in chromosome alignment and spindle bipolarity were observed in mitotic tumor cells, supporting MLN8237 distribution to, and AAK inhibition within tumor tissue. The AAK-selective activity of MLN8237 was also corroborated by an increase in the frequency of mitotic (pHistH3-positive) cells in posttreatment biopsies, and by an accumulation of...
cells with misaligned chromosomes and improperly formed bipolar spindles, phenotypes characteristic of selective AAK inhibition (1, 11, 14, 36). In contrast, as pHisT3 is a substrate of ABK, a treatment with a dominant effect of inhibition of ABK would be predicted to result in a decreased frequency of pHisT3-positive cells (1, 25, 44). Viewed collectively, the data from skin and tumor biopsies indicate that MLN8237 selectively inhibits AAK, but not ABK, in skin and tumor tissue, using the doses and schedules evaluated for pharmacodynamic effects in this study.

The inhibition of AAK activity by MLN8237 and its associated effects on mitosis indicate that MLN8237 may have a greater potential for antitumor activity in highly proliferative malignancies. While the primary goal of this phase I study was not to evaluate antitumor activity, MLN8237 treatment was associated with prolonged stable disease in several patients. Twenty-two (37%) patients achieved a best response of stable disease, which was durable for more than 6 months in 6 patients and for approximately 1 year or longer in 4, results suggestive of general tolerability to repeat treatment cycles. Objective antitumor activity was evident in one patient with a treatment-resistant pleomorphic liposarcoma; however, the observed 25.7% reduction in tumor diameter could not be classified as a response per RECIST. The antitumor activity associated with MLN8237 administration will require further investigation in appropriately designed phase II studies.

In conclusion, MLN8237 represents a novel class of anticancer agent designed for the selective inhibition of AAK. The pharmacokinetic data obtained here support the achievement of pharmacologically active plasma and tissue concentrations of MLN8237. Steady-state plasma exposures were achieved within 7 days of twice daily dosing, and correlative laboratory studies indicated robust pharmacodynamic effects reflecting AAK inhibition in both skin and tumor. The safety profile is consistent with AAK inhibition in proliferative tissues, including bone marrow, gastrointestinal tract, and hair follicles. Taken together with the pharmacokinetic and pharmacodynamic data, these results support a RP2D of 50 mg twice daily for 7 days in 21-day cycles in adults with advanced solid tumors. Building on the results of this study and those of another phase I trial that identified the same MTD and RP2D (Dees and colleagues, ref. 47; and manuscript submitted), the safety and antitumor activity of MLN8237 are being investigated for the treatment of a variety of malignancies in multiple phase II trials. In addition, on the basis of the findings of a phase II study in aggressive non-Hodgkin lymphoma (NCT00807495; ref. 49), single-agent MLN8237 is currently being investigated compared with investigator’s choice of pralatrexate, romidepsin, or gemcitabine in a randomized phase III trial in peripheral T-cell lymphoma (NCT01482962).

Disclosure of Potential Conflicts of Interest
J. Ecsedy, K. Venkatashrishnan, J. Jung, and H. Fingert have employment in Millennium. J. Tabernero is a Consultant/Advisory Board member for Millennium. No potential conflicts of interest were disclosed by the other authors.

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