Phase I Study of Aurora A Kinase Inhibitor MLN8237 in Advanced Solid Tumors: Safety, Pharmacokinetics, Pharmacodynamics, and Bioavailability of Two Oral Formulations

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Abstract

Purpose: This phase I study evaluated the safety, pharmacokinetics, pharmacodynamics, and efficacy of the investigational oral drug MLN8237 (alisertib), a small-molecule Aurora A kinase (AAK) inhibitor, in 87 adult patients with advanced solid tumors.

Experimental Design: Sequential cohorts of patients received MLN8237 5 to 150 mg orally once daily or twice daily for 7, 14, or 21 days, followed by 14 days' rest per cycle. MLN8237 pharmacokinetics was characterized, and the relative bioavailability of an enteric-coated tablet (ECT) formulation was evaluated in reference to the original powder-in-capsule (PIC) formulation. Pharmacodynamic effects of MLN8237 on inhibition of AAK activity were evaluated in skin biopsies. Tolerability and response to treatment were assessed.

Results: Common toxicities included fatigue, nausea, and neutropenia. Plasma exposures increased dose proportionally (5–150 mg/d), and were similar for PIC and ECT. The terminal half-life was 23 hours. At the maximum tolerated dose of 50 mg twice daily on the 7-day schedule, the mitotic index of the skin basal epithelium was increased within 24 hours after MLN8237 administration on days 1 and 7, a finding consistent with AAK inhibition. One (1%) patient achieved a partial response lasting for more than 1 year and received MLN8237 for 51 cycles; 20 (23%) patients achieved stable disease for ≥3 months.

Conclusions: This first-in-human trial of MLN8237 showed tolerability and favorable pharmacokinetics in this patient population. The recommended phase II dose of MLN8237 is 50 mg twice daily orally for 7 days in 21-day cycles, which is being evaluated further in the treatment of various solid tumors and hematologic malignancies. Clin Cancer Res; 1–10. ©2012 AACR.

Introduction

The Aurora family of serine/threonine protein kinases plays a critical role in the regulation of chromosomal segregation and cytokinesis during mitotic progression. Aurora A kinase (AAK) is expressed in all somatic cells undergoing mitotic cell division (1) and is thought to have an important role in oncosogenesis and tumor progression.

The AAK gene is amplified and/or overexpressed in many solid tumor types, including bladder cancer, breast cancer, colon cancer, head and neck cancer, non–small cell lung cancer (NSCLC), ovarian cancer, and pancreatic cancer, as well as hematologic malignancies (2–13). AAK overexpression has been associated with centrosome amplification and aneuploidy—a characteristic phenotype of tumor cells...
Translational Relevance

Although conventional antimitotic agents that perturb microtubule dynamics have shown therapeutic value in various malignancies, development of newer classes of antimitotic agents, including those that target enzymes involved in mitotic progression, might allow for more convenient administration, more manageable toxicity, and improved outcomes. MLN8237 (alisertib) is an oral, small-molecule inhibitor of Aurora A kinase (AAK)—a key mitotic regulator. This phase I study evaluated the safety, pharmacokinetics, pharmacodynamics, and antitumor activity of MLN8237 in 87 patients with advanced solid tumors. A recommended phase II dose was established for the powder-in-capsule (PIC) formulation, and a crossover bioavailability substudy confirmed similar absorption and exposure of a new enteric-coated tablet compared with PIC. Pharmacodynamic studies showed an accumulation of mitotic cells within skin biopsies, consistent with AAK inhibition. One patient achieved partial response and 20 achieved stable disease for ≥3 months. Further evaluation of MLN8237 is ongoing in various solid tumors and hematologic malignancies.

(9). Furthermore, AAK overexpression in vitro results in the transformation of normal cells (2, 8, 14). Inhibition of AAK by gene mutation, RNA interference, antibody microinjection, or ATP-competitive small-molecule kinase inhibitors results in a diverse array of mitotic progression defects, leading to cell death or mitotic arrest (14–20). For these reasons, AAK is a key focus of anticancer drug development.

The investigational oral agent MLN8237 (alisertib; refs. 21, 22) is a selective, small-molecule inhibitor of AAK (23) that was developed from the predecessor molecule MLN8054, for the treatment of advanced malignancies. MLN8237 is an ATP-competitive and reversible inhibitor of AAK with an inhibition constant (Ki) of 0.43 nmol/L (Millennium Pharmaceuticals, Inc. data on file), and was designed to minimize the benzodiazepine-like effects seen with MLN8054 (24). In preclinical studies, MLN8237 has showed both in vitro and in vivo activity in a broad range of tumor types (21, 22, 25–28). In vitro, MLN8237 showed antiproliferative activity across a broad range of both solid tumor and lymphoma cell lines (22). In mice bearing HT-116 colon tumor xenografts, MLN8237 administration resulted in increased mitotic index (MI), as well as dose-dependent reductions in bipolar mitotic spindles and chromosome alignment, supporting selective inhibition of AAK by MLN8237. Furthermore, MLN8237 inhibited tumor growth in solid tumor xenograft models, as well as causing regression in in vivo lymphoma models (22). In vivo preclinical studies also confirmed the expected toxicities of MLN8237 (including myelosuppression and gastrointestinal mucosa damage), which are consistent with the antimitotic action of the drug (Millennium Pharmaceuticals, Inc., data on file).

This phase I, dose-escalation clinical trial (NCT00500903) was the first study designed to investigate the safety, pharmacokinetics, pharmacodynamics, and antitumor activity of orally administered MLN8237 in adult patients with advanced solid tumors.

Materials and Methods

Study design

The primary objective of this phase I, open-label, dose-escalation study was to determine the maximum tolerated dose (MTD) and dose-limiting toxicities (DLT) of MLN8237 when administered orally for a minimum of 7 days and a maximum of 21 days, followed by a 14-day recovery period in each cycle. Secondary objectives included evaluation of single- and multiple-dose pharmacokinetics, assessment of pharmacodynamic effects as measured by AAK inhibition in proliferating skin cells, measurement of the relative bioavailability of a new enteric-coated tablet (ECT) formulation in reference to the original powder-in-capsule (PIC) formulation, and determination of the antitumor activity of MLN8237.

The structure of MLN8237 has been published previously (21, 22). MLN8237 was administered orally for 7, 14, or 21 consecutive days, followed by a 14-day recovery period, in 21-, 28-, or 35-day cycles. Sequential cohorts of 3 to 6 patients received escalating doses of the MLN8237 PIC formulation following a standard 3þ3 phase I trial design to define the MTD, recommended phase II dose (RP2D), and preferred dosing schedule. MLN8237 was administered with 8 oz of water, with patients receiving nothing by mouth except for water and prescribed medications for 2 hours before and 1 hour after each dose. All patients received repeated cycles of MLN8237 treatment, provided that treatment was tolerable and their disease did not progress.

The 7-day dosing schedule was evaluated first. The starting dose for human clinical testing was 5 mg/d, one sixth of the highest nonseverely toxic dose in dogs (Millennium Pharmaceuticals, Inc., data on file). Thus, the first patient cohort received MLN8237 PIC 5 mg/d once daily for 7 days, which was then doubled in successive cohorts until 2 or more patients experienced a grade 2 drug-related toxicity or 1 patient experienced a grade ≥3 MLN8237-related toxicity. Subsequently, dose escalation continued at no more than a 40% increment until the MTD was established. The MTD was defined as the highest dose level at which DLTs occurred in 1 or fewer of the first 6 treated patients. After the finding that once daily dosing at higher dose levels was associated with somnolence, twice daily dosing was explored. Upon determining the 7-day MTD, additional patients were treated on the 7-day schedule to better define safety, pharmacokinetics, and pharmacodynamics. Longer dosing durations were also evaluated. Using a starting dose equivalent to 50% of the 7-day MTD, 14-day once daily dosing was explored in additional cohorts. Upon determining that once daily dosing on the 14-day schedule was tolerable, further dose-escalation cohorts (3–6 patients)
were enrolled to evaluate safety on a 21-day dosing schedule.

The study was conducted at 3 sites in the United States. Independent review boards at all participating institutions approved the study, which was conducted according to the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice. All patients provided their written informed consent for study participation.

Relative bioavailability substudy

During preclinical studies, MLN8237 was found to have reduced solubility in acidic solution, and accordingly, MLN8237 was initially formulated for early investigational use as a buffered PIC. An ECT formulation of the drug was subsequently developed to bypass the stomach and delay dissolution until delivery to the upper small bowel. In this relative bioavailability substudy, the tolerability of the ECT formulation was evaluated with twice daily dosing in a 7-day treatment schedule with accelerated titration. In these dose-escalation cohorts, 1 patient was enrolled per dose level (starting ECT dose: 10 mg twice daily) with rapid dose doubling up to 40 mg twice daily (Supplementary Fig. S1). Subsequently, the relative bioavailability of the ECT in reference to the PIC formulation was characterized in a 2-cycle, 2-way crossover pharmacokinetic study design (Supplementary Fig. S2). In separate cohorts, a total of 14 patients received either the ECT or PIC formulations (40 mg twice daily on the 7-day schedule), followed by crossover to the other formulation in the next cycle.

Patients

Eligible patients were aged ≥18 years, and had histologically or cytologically confirmed metastatic and/or advanced solid tumors (including lymphomas) refractory to standard therapy, or for which no effective standard treatment was available. Other inclusion criteria included an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and radiographically or clinically evaluable disease. Eligible patients were required to have adequate organ function, including an absolute neutrophil count (ANC) of ≥1,500 cells/mm³; platelet count of ≥100,000/mm³; serum creatinine of ≤1.6 mg/dL or a measured or estimated (Cockcroft–Gault formula) creatinine clearance of ≥40 ml/min; bilirubin of ≤1.5 × upper limit of normal (ULN); aspartate aminotransferase (AST) or alanine aminotransferase of ≤2.5 × ULN; alkaline phosphatase (ALP) of ≤2.5 × ULN (AST and ALP levels ≤5 × ULN were permitted if reasonably ascribed to liver or bone metastases); and a left ventricular ejection fraction (LVEF) of ≥50%. Patients could not have received more than 4 prior cytotoxic chemotherapeutic regimens, prior stem cell transplant, or prior radiation therapy involving ≥25% of hematopoietically active bone marrow. Patients with active central nervous system (CNS) metastases, impaired gastrointestinal absorption, sleep apnea, clinically serious infection, or clinically significant electrocardiography abnormalities were excluded.

Assessments

Safety assessments. DLTs were defined during cycle 1 by the following MLN8237-related events: grade 4 neutropenia (ANC <500 cells/mm³) lasting ≥7 consecutive days, or with fever and/or infection; a confirmed platelet count of <25,000/mm³; grade ≥3 nausea, emesis, or diarrhea, despite maximal supportive therapy; any other grade ≥3 nonhematologic toxicity (excluding grade 3 arthralgia/myalgias, and brief [<1 week] grade 3 fatigue); treatment delay of >1 week because of a failure of adequate hematologic or nonhematologic recovery from the previous cycle; or any other MLN8237-related grade ≥2 nonhematologic toxicity that, in the opinion of the investigator, required dose reduction/discontinuation. The MTD was defined as the highest dose cohort in which fewer than 2 of 6 patients experienced DLTs. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (29). The final RP2D integrated the formal MTD with an assessment of cumulative safety data from all cohorts on all evaluated schedules.

Pharmacokinetic assessments. Blood samples were drawn at baseline and at serial time points after MLN8237 dosing for measurement of plasma MLN8237 concentrations. In the dose-escalation cohorts, pharmacokinetic sampling was conducted over a 24 (once daily dosing) or 12 hours (twice daily dosing) period following the first dose on day 1 of cycle 1, and following multiple-dose administration (day 7 for the 7-day schedule, days 7 and 14 for the 14-day schedule, and days 7 and 21 for the 21-day schedule). Additional pharmacokinetic samples were collected for up to 120 or 144 hours following the last dose of MLN8237 in cycle 1 to estimate terminal half-life (t1/2). In the relative bioavailability substudy, pharmacokinetic samples were collected over the day 7 dosing period in cycles 1 and 2 to permit estimation of steady-state systemic exposure of the ECT in reference to the PIC formulation. Urine was collected over 24 hours postdose on day 1 in all dose-escalation cohorts to estimate renal clearance.

Full details pertaining to the pharmacokinetic sampling schedules and analytical methods are provided in the Online Supplementary Information.

Pharmacodynamic assessments. Pharmacodynamic effects of MLN8237 administration on inhibition of AAK activity were evaluated by measuring the change in the frequency of mitotic cells in the basal epithelium of the skin following treatment. During dose escalation, serial 2 to 4 mm skin punch biopsies were obtained and prepared as previously described (25, 30, 31). Patients had biopsies conducted predose and at 6 and 24 hours after the first dose. Several patients at higher dose levels had biopsies at the 6 hours time point on day 7 and day 21. Sections (5 μm) of formalin-fixed, paraaffin-embedded skin punch biopsies were deparaffinized and stained by standard methods with hematoxylin and eosin using a Leica Autostainer XL (Meyer Instruments, Inc.). Immunofluorescent staining for phosphohistone H3 (pHistH3) and mitotic protein monoclonal #2 (MPM2) was used to identify mitotic cells, and the MI was calculated as the number of mitotic cells per millimeter
length of the basal epithelial layer as described previously (25).

Clinical and response evaluation. Pretreatment evaluation included a medical history, physical examination, assessment of ECOG performance status, measurement of any tumor markers in blood, and assessment of baseline disease status via computed tomography or MRI. In a subset of patients, LVEF was measured serially at baseline, at the end of cycle 1, and at study end using multigated radionucleotide scans or echocardiography. Tumor response was measured at the end of every 2 cycles and at the end-of-study/treatment visit, according to RECIST (32).

Statistical analyses
Descriptive statistics was used to summarize patients’ baseline characteristics, MLN8237 efficacy and safety measurements, and pharmacokinetic and pharmacodynamic parameters. For the relative bioavailability study, the ratios of geometric mean day 7 area under the concentration–time curve from time 0 to t \( [\text{AUC}(0-t)] \) and maximum serum concentration \( (C_{\text{max}}) \) of ECT relative to PIC and associated 2-sided 90% confidence intervals (CI) were calculated using mixed-effects models.

Results
Patient demographics and baseline characteristics
A total of 87 patients received MLN8237 treatment; of these, 65 received the PIC formulation and 22 the ECT formulation, including 14 who were evaluable in the relative bioavailability study. Patient demographics and disease characteristics are summarized in Table 1. The median age was 60 years (range, 36–83) and 51% of patients were male. The most common tumor types were colorectal cancer \( (n = 15, 17\%) \), NSCLC \( (n = 10, 11\%) \), and ovarian cancer \( (n = 10, 11\%) \). All but 2 patients had received at least 1 prior systemic therapy; 60 (69%) patients had received 3 or more prior systemic therapies.

DLTs and determination of MTD
DLTs associated with MLN8237 treatment during the first treatment cycle are summarized by dose and scheduled in Table 2. DLTs were first observed in 3 of 6 patients treated at 150 mg once daily on the 7-day schedule, and included grade 2 somnolence, grade 2 confusion, grade 3 stomatitis, grade 3 asthenia, and grade 4 neutropenia. Among 6 patients treated at the reduced dose level of 110 mg once daily, 1 patient experienced transient dose-limiting grade 3 CNS events of somnolence, confusion, and memory impairment; 3 other patients had transient grade 1 or 2 somnolence, but these were not considered to be DLTs. CNS toxicities typically occurred within the first few days of dosing in these patients and prevented completion of the treatment cycle. As the sedative effects of GABA\(_A\) receptor agonists (e.g., benzodiazepines) are generally thought to be related to peak plasma concentrations (33), twice daily dosing was therefore explored in subsequent cohorts, with the goal of reducing \( C_{\text{max}} \) and improving tolerability with regard to CNS events, while preserving total daily systemic exposures (AUC). At the 60 mg twice daily dose on the 7-day schedule, 2 of 6 patients experienced DLTs during the first treatment cycle (1 patient with grade 4 febrile neutropenia and 1 patient with grade 4 thrombocytopenia), whereas only 1 of the first 6 patients treated at the 50 mg twice daily dose level experienced DLTs (grade 4 febrile neutropenia and grade 4 thrombocytopenia). Thus, 50 mg twice daily was determined as the MTD on the 7-day schedule. The 50 mg twice daily cohort was subsequently expanded to a total of 11 patients, and a further DLT (grade 4 febrile neutropenia) was observed in 1 patient.

The tolerability of MLN8237 administered in 14- and 21-day dosing schedules was subsequently investigated. On the 14-day schedule, no DLTs were reported in 3 patients who received 25 mg once daily. Twice daily dosing with 40 mg for 14 days was explored and found to be intolerable with 2 patients experiencing DLTs (grade 3 diarrheea, grade 3 febrile neutropenia, and grade 4 thrombocytopenia). As predefined in the study protocol, dosing over a 21-day period was then evaluated. No DLTs were reported at 25 mg once daily \( (n = 3) \) or 50 mg once daily \( (n = 6) \). Among 7 patients who received 70 mg once daily on the 21-day schedule, 6 of whom were evaluable, 1 patient experienced DLTs including grade 3 diarrheea, grade 4 neutropenia, grade 4 thrombocytopenia, and

<p>| Table 1. Patient baseline demographics and disease characteristics |
|-------------------|----------|</p>
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N = 87</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, y (range)</td>
<td>60 (36–83)</td>
</tr>
<tr>
<td>Male/female, n (%)</td>
<td>44 (51)/43 (49)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td>75 (86)</td>
</tr>
<tr>
<td>Black or African-American</td>
<td>11 (13)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Tumor type, n (%)</td>
<td>15 (17)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>10 (11)</td>
</tr>
<tr>
<td>NSCLC</td>
<td>10 (11)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>8 (9)</td>
</tr>
<tr>
<td>Soft tissue sarcoma</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Head and neck cancer</td>
<td>38 (44)</td>
</tr>
<tr>
<td>Other a</td>
<td>42 (48)/45 (52)</td>
</tr>
<tr>
<td>ECOG performance status 0/1, n (%)</td>
<td>85 (98)</td>
</tr>
<tr>
<td>Systemic therapy</td>
<td>49 (56)</td>
</tr>
<tr>
<td>Radiation</td>
<td>60 (69)</td>
</tr>
<tr>
<td>≥3 prior systemic therapies, n (%)</td>
<td>11 (13)</td>
</tr>
<tr>
<td>aAnal carcinoma ( (n = 3) ), breast cancer ( (n = 3) ), cervical carcinoma ( (n = 1) ), esophageal cancer ( (n = 1) ), gallbladder carcinoma ( (n = 2) ), intestinal cancer ( (n = 3) ), liver cancer ( (n = 1) ), mesothelioma ( (n = 2) ), pancreatic carcinoma ( (n = 4) ), prostate cancer ( (n = 4) ), skin cancer ( (n = 1) ), small cell lung cancer ( (n = 4) ), uterine carcinoma ( (n = 4) ), tumors of unknown origin ( (n = 5) )</td>
<td></td>
</tr>
</tbody>
</table>
grade 3 cardiac dysfunction requiring intensive care. Although only 1 of 7 patients experienced DLTs in cycle 1, the investigators elected not to escalate further, given the severity of these DLTs, as well as the CNS toxicity seen earlier with escalation to 110 mg once daily on the 7-day schedule. In addition, of 9 patients who received total daily doses exceeding 50 mg in the 14- and 21-day schedules, 6 required doses to be held for AEs that occurred during the first 2 treatment cycles. Patients on the 14- and 21-day schedules frequently had prolonged blood count recovery times that delayed the start of subsequent cycles. Assessment by total dose per cycle revealed that with a total cycle dose of more than 700 mg, a higher number of patients required dose delay or reduction because of AEs in the first 2 cycles.

After integrating all safety data, a decision was made to abandon dosing schedules exceeding 7 days’ duration, and to declare 50 mg twice daily on the 7-day schedule as the RP2D.

Adverse events

All patients reported at least 1 treatment-emergent adverse event, and 47 (54%) patients reported at least 1 grade ≥3 adverse event during the study. Drug-related adverse events are summarized in Table 3. The most common drug-related adverse events (all grades) were fatigue (n = 40, 46%), nausea (n = 40, 46%), and neutropenia (n = 37, 43%). Thirty-eight (44%) patients experienced at least 1 drug-related grade ≥3 adverse event (Table 3); the most common was neutropenia (n = 26, 30%). Drug-related grade ≥3 febrile neutropenia was reported in 2 of 11 patients at the RP2D of 50 mg twice daily on the 7-day schedule. Drug-related peripheral neuropathy was not a significant or frequent finding reported in only 2 patients who received doses above the MTD (1 at 110 mg once daily on the 7-day schedule; 1 at 60 mg twice daily on the 7-day schedule). CNS disorders, including dizziness and somnolence, were reported in 5 of 11 patients treated at the RP2D of 50 mg twice daily on the 7-day schedule; however, these events were generally limited to grade 1/2 severity and resolved during the treatment-free period without frequent requirement for dose modification or supportive care.

Significant cumulative toxicities have not been evident to date. Ten (11%) patients discontinued because of adverse events, including febrile neutropenia, neutropenia, and thrombocytopenia. One patient receiving 70 mg once daily on the 21-day schedule developed symptomatic left ventricular (LV) dysfunction. This patient’s cardiac event was complicated by pericardial involvement due to synovial sarcoma and before treatment with doxorubicin and thoracic radiotherapy, thus confounding a possible relationship to MLN8237. Significant LV dysfunction was not observed in subsequent patients, including several treated for periods exceeding 6 months. There were 4 (5%) on-study deaths within 30 days of the last dose of study drug, none of which were deemed to be drug related.

Table 2. Summary of MLN8237-related DLTs during cycle 1, by dose and schedule

<table>
<thead>
<tr>
<th>Dose and schedule</th>
<th>Number of patients treated</th>
<th>Number of patients with DLTs</th>
<th>Type and grade of DLTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-d schedule</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mg q.d.</td>
<td>3</td>
<td>0</td>
<td>Somnolence, confusion, memory impairment (all grade 3)</td>
</tr>
<tr>
<td>10 mg q.d.</td>
<td>3</td>
<td>0</td>
<td>Somnolence (grade 2), confusion (grade 2), stomatitis (grade 3), asthenia (grade 3), neutropenia (grade 4)</td>
</tr>
<tr>
<td>20 mg q.d.</td>
<td>3</td>
<td>0</td>
<td>Febrile neutropenia (grade 4), thrombocytopenia (grade 4)</td>
</tr>
<tr>
<td>40 mg q.d.</td>
<td>3</td>
<td>0</td>
<td>Febrile neutropenia (grade 4), thrombocytopenia (grade 4)</td>
</tr>
<tr>
<td>80 mg q.d.</td>
<td>3</td>
<td>0</td>
<td>Diarrhea (grade 3), febrile neutropenia (grade 3), thrombocytopenia (grade 4)</td>
</tr>
<tr>
<td>110 mg q.d.</td>
<td>6</td>
<td>1</td>
<td>Diarrhea (grade 3), thrombocytopenia (grade 4), ventricular dysfunction (grade 3), neutropenia (grade 4)</td>
</tr>
<tr>
<td>150 mg q.d.</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>50 mg b.i.d.</td>
<td>11</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>60 mg b.i.d.</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>14-d schedule</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mg q.d.</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>40 mg b.i.d.</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>21-d schedule</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mg q.d.</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>50 mg q.d.</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>70 mg q.d.</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: q.d., once daily; b.i.d., twice daily.
Table 3. Drug-related adverse events of all grades (incidence >10%), and corresponding rates of grade ≥3 adverse events (or those with an incidence >5%) observed in patients treated with MLN8237

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>N = 87</th>
<th>All grades</th>
<th>Grade ≥3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with at least one drug-related adverse event, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>40 (46)</td>
<td>3 (3)</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>40 (46)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>37 (43)</td>
<td>26 (30)</td>
<td></td>
</tr>
<tr>
<td>Alopecia</td>
<td>36 (41)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Somnolence</td>
<td>31 (36)</td>
<td>2 (2)</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>29 (33)</td>
<td>6 (7)</td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>28 (32)</td>
<td>7 (8)</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>23 (26)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Anorexia</td>
<td>21 (24)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Stomatitis</td>
<td>21 (24)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>10 (11)</td>
<td>5 (6)</td>
<td></td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>7 (8)</td>
<td>7 (8)</td>
<td></td>
</tr>
</tbody>
</table>

Pharmacokinetics

Pharmacokinetic data were available in 83 patients who received the PIC formulation. A summary of pharmacokinetic parameters for MLN8237 by dose and schedule is provided in Supplementary Table S1. Figure 1 shows the mean plasma concentration–time profiles for patients receiving MLN8237 PIC 5 to 150 mg once daily (A) and 50 or 60 mg twice daily (B) on the 7-day schedule. On the basis of an integrated assessment of pharmacokinetic data across the dosing cohorts, we concluded that MLN8237 absorption was fast with an overall median time from dosing to first occurrence of maximum serum concentration (Tmax) of 2 hours (range, 1–6) postdose. Mean steady-state T1/2 following multiple dosing was approximately 23 hours [coefficient of variation (CV): 77%]. Pharmacokinetic steady-state conditions were consistently achieved by approximately day 7 following daily oral administration on the basis of similar mean AUC0–<i>∞</i> values between days 7 and 14 in patients on the 14-day dosing schedule. The overall mean peak/trough ratios were 2.6 and 5.0 for twice daily and once daily dosing, respectively, with corresponding mean accumulation ratios of 2.5 and 1.8 for twice daily and once daily dosing, respectively.

The steady-state exposure of MLN8237 increased approximately dose proportionally over the 5 to 150 mg once daily dose range (Fig. 2A, n = 69), supported by the lack of dose dependence of the steady-state apparent oral clearance (CL<sub>app</sub>/F; Fig. 2B, n = 69). At the RP2D of 50 mg twice daily on the 7-day schedule, the geometric mean steady-state average concentration (C<sub>ss,avg</sub>) was 2.7 μM (CV: 41%, n = 10). Negligible urinary excretion of MLN8237 was observed. The renal clearance of MLN8237 (geometric mean 0.8 mL/h, n = 36) was less than 0.1% of the apparent oral clearance (geometric mean 4.45 L/h, n = 69).

Relative bioavailability substudy: ECT versus PIC

Twenty-two patients received the ECT formulation: 10 mg twice daily and 20 mg twice daily (each n = 1) and 40 mg twice daily (n = 20). Fourteen patients completed the protocol-specified dosing and were evaluable. The steady-state relative bioavailability of the ECT formulation in reference to PIC was 90% (90% CI, 74.4–108.8). Median T<sub>max</sub> was 2.4 hours for ECT and 2 hours for PIC. The mean accumulation ratio of MLN8237 administered twice daily as ECT was approximately 2.8-fold, and the mean peak/trough ratio was approximately 2.5, consistent with the values determined for twice daily dosing with PIC.

Pharmacodynamics

Pre- and post-dose skin biopsies for assessment of the pharmacodynamic effects of MLN8237 on AAK inhibition were available from 58 of 59 patients in the dose-escalation portion of the study who received the PIC formulation. A posttreatment increase in skin MI, as showed by an increase in the number of pHistH3- and MPM2-positive mitotic cells in the basal epithelial layer of the skin compared with the number observed in pretreatment biopsies, was observed in most patients who received MLN8237; this phenotype is consistent with inhibition of AAK by MLN8237. The magnitude of the increase was greatest in the higher dose cohorts from biopsies collected 24 hours after the day 1 dose and 6 hours after the day 7 dose, including patients receiving the RP2D of 50 mg twice daily (Fig. 3).

Response and duration of treatment

Across all doses and schedules, patients received a median of 2 treatment cycles (range, 1–51). Fourteen (16%) patients continued treatment for ≥6 cycles. One patient with progressive, platinum- and radiation–refractory ovarian cancer achieved a confirmed partial response (PR) lasting for more than 1 year; this patient continued on MLN8237 treatment for 51 cycles over a period of 2.9 years (Supplementary Fig. S3). An additional 33 (38%) patients achieved a best response of stable disease, with 20 (23%) achieving stable disease lasting ≥3 months; of these, 2 patients, 1 with skin cancer and 1 with NSCLC (bronchioalveolar carcinoma), achieved prolonged stable disease lasting 21.9 and 24.5 months, respectively.

Discussion

Antimitotic therapeutic agents that directly disrupt normal microtubule dynamics, including the taxanes, vinca alkaloids, and epothilones (34), are among the most widely used classes of drugs for the treatment of cancer. These agents have showed therapeutic value in a diverse array of malignancies as single agents and in combination with other drugs. More recently, considerable efforts have
focused on identifying alternative approaches for perturbing mitotic machinery by selectively targeting enzymes that are key regulators of normal mitotic progression. Several enzyme classes have been identified as targets for this drug development strategy including kinases, kinesins, and GTPases. It is thought that these newer antimitotic strategies will provide advantages over existing antimicrotubule agents, including more convenient administration and dosing regimens, more manageable toxicity, and improved outcomes. The Aurora kinases, and AAK in particular, are examples of next-generation antimitotic targets for cancer therapy.

This study was the first-in-human evaluation of the oral, AAK inhibitor MLN8237 in patients with advanced solid tumors. Dose escalation was conducted with schedules of varying duration, with characterization of the pharmacokinetics and pharmacodynamics of the PIC formulation, and determination of the relative bioavailability of the ECT formulation in reference to the PIC. For the MLN8237 PIC formulation, the MTD and RP2D were determined to be...
50 mg twice daily for 7 days in 21-day cycles. Higher cumulative doses per cycle and dosing schedules of longer duration (14 and 21 days) were not well tolerated.

At the RP2D, MLN8237 was generally well tolerated in this patient population. The most common adverse events were fatigue, nausea, and neutropenia, which likely represent on-target toxicities related to the AAK inhibitory activity of the drug in highly proliferative tissues. These adverse events were generally reversible in the recovery period between dosing intervals, and significant cumulative toxicities were not observed. The toxicity profile described here is similar to that of other Aurora kinase inhibitors currently under clinical evaluation (35).

MLN8237 and its precursor molecule MLN8054 are structurally related to the benzodiazepine family (22, 33). In this study, the frequency of benzodiazepine-associated CNS toxicities appeared to be reduced when MLN8237 was administered in divided (twice daily) doses on the 7-day schedule; this is likely to be related to the finding that twice daily dosing reduced peak plasma concentrations while maintaining overall systemic exposure. Unlike our experience with MLN8054 (24), MLN8237-related grade ≥3 CNS events were generally limited to doses exceeding the MTD.

MLN8237 was absorbed rapidly and steady state was achieved within 1 week of dosing, consistent with an estimated mean $t_{1/2}$ of 23 hours. Over the dose range of 5 to 150 mg/d evaluated in this study, MLN8237 steady-state exposures increased approximately dose proportionally. Approximately 2.5-fold accumulation was observed with twice daily dosing, with a moderate level of fluctuation in plasma concentrations of approximately 2.5-fold over the steady-state dosing interval. The steady-state average plasma concentrations achieved with the RP2D of 50 mg twice daily (2.7 μM) exceeded the estimated efficacious steady-state plasma concentration of approximately 1 μM, which was consistently associated with antitumor activity in preclinical xenograft models (36). This study also shows that the bioavailability and pharmacokinetic properties of MLN8237 formulated as PIC or ECT are similar, providing pharmacokinetic support for transition to ECT in future clinical development. It is of note that in a subsequent dose-escalation study of MLN8237 ECT in adult patients with advanced solid tumors, the RP2D was shown to be 50 mg.

Figure 3. Mitotic index in patients with evaluable pre-dose and day 1 6 hours (A), day 1 24 hours (B), day 7 6 hours (C), and day 21 6 hours (D) postdose skin biopsies.
twice daily for 7 days in repeat 21-day cycles (37), consistent with the relative bioavailability of ECT versus PIC in this study.

The pharmacodynamic effects of MLN8237 in inhibiting AAK activity were reflected in an accumulation of pHistH3- and MPM2-immunopositive mitotic cells in skin biopsies. This effect was observed by 24 hours postdosing on the first day of treatment and after steady-state plasma concentrations of MLN8237 were achieved on day 7. The magnitude of the pharmacodynamic response was greater for patients in the higher dose cohorts. The selectivity of the inhibitory activity of MLN8237 for AAK, rather than pan-Aurora kinases or Aurora B kinase (ABK) was confirmed here through observation of an increase in the frequency of pHistH3-immunopositive cells in posttreatment biopsies. As ABK catalyzes the phosphorylation of pHistH3 (25), inhibition of ABK would have resulted in a posttreatment decrease in the frequency of pHistH3-positive cells (21, 33, 38).

It is possible that the posttreatment change in the skin MI would have been more pronounced later in the dosing schedule. Only a few of our patients had biopsies after 7 days of dosing. A parallel phase I study in Europe that defined the same MLN8237 MTD and schedule showed maximal and consistent pharmacodynamic effects in skin and tumor after 7 days of dosing when steady-state plasma concentrations of MLN8237 had been reached (ref. 39 and Cervantes and colleagues, manuscript submitted). Together, these data show that MLN8237 inhibits AAK in both skin and tumor at the tolerated doses, including the RP2D of 50 mg twice daily.

MLN8237 treatment was associated with antitumor activity in some heavily pretreated patients. One patient with platinum- and radiation-refractory ovarian cancer achieved a durable PR by RECIST and continued treatment for 51 cycles over a period of 2.9 years without evidence of cumulative toxicities. Twenty (23%) patients achieved stable disease lasting ≥3 months; of these, 2 patients achieved prolonged stable disease of 21.9 months and 24.5 months, respectively. The preliminary results reported in this phase I study suggest that MLN8237 may provide durable disease control lasting 3 months; of these, 2 patients achieved prolonged stable disease of 21.9 months and 24.5 months, respectively. The preliminary results reported in this phase I study suggest that MLN8237 may provide durable disease control in some patients with advanced solid tumors, and the safety profile indicates that prolonged treatment can be tolerable with appropriate monitoring and supportive care. In conclusion, the RP2D for MLN8237 is 50 mg twice daily for 7 days followed by a 14-day recovery period, in 21-day cycles. MLN8237 was generally tolerable in patients with advanced malignancies, with the most common toxicities observed in highly proliferative tissues and likely related to AAK inhibition. Pharmacodynamic effects in the skin were also consistent with AAK inhibition. The pharmacokinetic and pharmacodynamic results, and the associated clinical toxicity profile, collectively support the achievement of bioactive and clinically relevant MLN8237 exposures. The relative bioavailability analyses also confirmed suitability of the ECT formulation for further clinical development. These results support further investigation of MLN8237 for treatment of patients with advanced malignancies. MLN8237 continues to be evaluated in phase I, II, and III clinical studies in a variety of solid tumors and hematologic malignancies.

Disclosure of Potential Conflicts of Interest
E.C. Dees has Research funding from Millennium Pharmaceuticals, Inc.; R.B. Cohen has research funding and consultancy or advisory role from Millennium Pharmaceuticals, Inc.; H. Liu, H. Fingert, K. Venkatakrishnan, and M. Manfredi have employment from Millennium Pharmaceuticals, Inc. No potential conflicts of interest were disclosed by the other authors.

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