Phase 1 Study of Aurora A Kinase Inhibitor MLN8237 in Advanced Solid Tumors: Safety, Pharmacokinetics, Pharmacodynamics, and Bioavailability of Two Oral Formulations

Running head: Phase 1 study of AAK inhibitor, MLN8237, in solid tumors

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Translational Relevance:

Although conventional antimitotic agents that perturb microtubule dynamics have demonstrated 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 1 study evaluated the safety, pharmacokinetics, pharmacodynamics, and antitumor activity of MLN8237 in 87 patients with advanced solid tumors. A recommended phase 2 dose was established for the powder-in-capsule (PIC) formulation, and a cross-over bioavailability sub-study confirmed similar absorption and exposure of a new enteric-coated tablet compared with PIC. Pharmacodynamic studies demonstrated an accumulation of mitotic cells within skin biopsies, consistent with AAK inhibition. One patient achieved PR and twenty achieved stable disease for ≥3 months. Further evaluation of MLN8237 is ongoing in various solid tumors and hematologic malignancies.
Abstract

Purpose: This phase 1 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 5–150 mg orally once-daily (QD) or twice-daily (BID) for 7, 14, or 21 days, followed by 14 days’ rest per cycle. MLN8237 pharmacokinetics were 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/day), and were similar for PIC and ECT. The terminal half-life was 23 h. At the MTD of 50 mg BID on the 7-day schedule, the MI of the skin basal epithelium was increased within 24 h after MLN8237 administration on days 1 and 7, a finding consistent with AAK inhibition. One (1%) patient achieved a partial response lasting >1 year and received MLN8237 for 51 cycles; 20 (23%) patients achieved stable disease for ≥3 months.

Conclusion: This first-in-human trial of MLN8237 demonstrated tolerability and favorable pharmacokinetics in this patient population. The recommended phase 2 dose of MLN8237 is 50 mg BID orally for 7 days in 21-day cycles, which is being evaluated further in the treatment of various solid tumors and hematologic malignancies.
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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 oncogenesis and tumor progression. The AAK gene is amplified and/or over-expressed in many solid tumor types, including bladder, breast, colon, head and neck, non-small cell lung, ovarian, and pancreatic cancer, as well as hematologic malignancies (2–13). AAK over-expression has been associated with centrosome amplification and aneuploidy—a characteristic phenotype of tumor cells (9). Furthermore, AAK over-expression \textit{in vitro} results in the transformation of normal cells (2, 8, 14). Inhibition of AAK by gene mutation, RNA interference, antibody microinjection, or adenosine triphosphate (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) (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 nM (Millennium Pharmaceuticals, Inc., data on file), and was designed to minimize the benzodiazepine-like effects seen with MLN8054 (24). In preclinical studies, MLN8237 has demonstrated both in-vitro and in-vivo activity in a broad range of tumor types (21, 22, 25–28). \textit{In vitro}, MLN8237 showed antiproliferative activity across a broad range of both solid tumor and lymphoma cell lines (22).
mice bearing HCT-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. Further, 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 1, 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 1, open-label, dose-escalation study was to determine the MTD and dose-limiting toxicities (DLTs) 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 three to six patients received escalating doses of the MLN8237 PIC formulation following a standard 3+3 phase I trial design in order to define the MTD, recommended phase 2 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 h before and 1 h 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/day, 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/day once-daily (QD) for 7 days, which was then doubled in successive cohorts until two or more patients experienced a grade 2 drug-related toxicity, or one 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 one or fewer of the first six treated patients. After the finding that QD dosing at higher dose levels was associated with somnolence, twice-daily (BID) 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 QD dosing was explored in additional cohorts. Upon determining that QD dosing on the 14-day schedule was tolerable, further dose-escalation cohorts (three to six patients) were enrolled to evaluate safety on a 21-day dosing schedule.

The study was conducted at three sites in the USA. 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 sub-study

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 sub-study, the tolerability of the ECT formulation was evaluated with BID dosing in a 7-day treatment schedule with accelerated titration. In these dose-escalation cohorts, one patient was enrolled per dose level (starting ECT dose: 10 mg BID) with rapid dose-doubling up to 40 mg BID (Supplementary Fig. S1). Subsequently, the relative bioavailability of the ECT in reference to the PIC formulation was characterized in a two-cycle, two-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 BID on the 7-day schedule), followed by cross-over 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) ≥1,500 cells/mm³, a platelet count ≥100,000/mm³, serum creatinine ≤1.6 mg/dL or a measured or estimated (Cockcroft–Gault formula) creatinine clearance of ≥40 mL/min, bilirubin ≤1.5 x upper limit of normal (ULN), AST or ALT aminotransferases ≤2.5 x ULN; alkaline phosphatase (ALP) ≤2.5 x ULN (AST and ALP levels <5 x 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 four prior cytotoxic chemotherapeutic regimens, prior stem cell transplant, or prior radiation therapy involving ≥25% of hematopoietically active bone marrow. Patients with active CNS metastases, impaired gastrointestinal absorption, sleep apnea, clinically serious infection, or clinically significant ECG 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 <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 two of six patients experienced DLTs. Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) 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
performed over a 24-h (QD dosing) or 12-h (BID 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 h following the last dose of MLN8237 in cycle 1 to estimate terminal half-life
\( t_{1/2} \). In the relative bioavailability sub-study, 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 h post-dose 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 2–4 mm skin punch biopsies were obtained
and prepared as previously described (25, 30, 31). Patients had biopsies performed
pre-dose and at 6 and 24 hours after the first dose. Several patients at higher dose
levels had biopsies at the 6 hour time point on day 7 and day 21. Sections (5 \( \mu m \)) of
formalin-fixed, paraffin-embedded skin punch biopsies were de-paraffinized and
stained by standard methods with hematoxylin and eosin using a Leica Autostainer
XL (Meyer Instruments, Inc.). Immunofluorescent staining for phospho-histone 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 mm length of the basal epithelial layer as described previously (25).

Clinical and response evaluation. Pre-treatment 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 (CT) or magnetic resonance imaging (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 (MUGA) or echocardiography. Tumor response was measured at the end of every two cycles and at the end-of-study/-treatment visit, according to RECIST (32).

Statistical analyses

Descriptive statistics were 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–t (AUC(0–t)) and maximum serum concentration (C_{max}) (ECT relative to PIC) and associated two-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 two patients had received at least one prior systemic therapy; 60 (69%) patients had received three 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 schedule in Table 2. DLTs were first observed in three of six patients treated at 150 mg QD on the 7-day schedule, and included grade 2 somnolence, grade 2 confusion, grade 3 stomatitis, grade 3 asthenia, and grade 4 neutropenia. Among six patients treated at the reduced dose level of 110 mg QD, one patient experienced transient dose-limiting grade 3 CNS events of somnolence, confusion, and memory impairment; three 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), BID 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 BID dose on the 7-day schedule, two of six patients experienced DLTs during the first treatment cycle (one patient with grade 4 febrile neutropenia and one patient with grade 4 thrombocytopenia), whereas only one of the first six patients treated at the 50 mg BID dose level experienced DLTs (grade 4 febrile neutropenia and grade 4 thrombocytopenia). Thus, 50 mg BID was determined as the MTD on the 7-day schedule. The 50 mg BID cohort was subsequently expanded to a total of 11 patients, and a further DLT (grade 4 febrile neutropenia) was observed in one 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 three patients who received 25 mg QD. BID dosing with 40 mg for 14 days was explored and found to be intolerable with two patients experiencing DLTs (grade 3 diarrhea, 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 QD ($n = 3$) or 50 mg QD ($n = 6$). Among seven patients who received 70 mg QD on the 21-day schedule, six of whom were evaluable, one patient experienced DLTs including grade 3 diarrhea, grade 4 neutropenia, grade 4 thrombocytopenia, and grade 3 cardiac dysfunction requiring intensive care. Although only one of seven 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 QD on the 7-day schedule. In addition, of nine patients who received total daily doses exceeding 50 mg in the 14- and 21-day schedules, six required doses to be held for AEs that
occurred during the first two treatment cycles. Patients on the 14- and 21-day
dates 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 >700 mg, a higher number of patients required dose delay or
reduction due to AEs in the first two cycles.

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

**Adverse events**

All patients reported at least one treatment-emergent AE, and 47 (54%) patients reported at least one grade ≥3 AE during the study. Drug-related AEs are
summarized in Table 3. The most common drug-related AEs (all grades) were
fatigue (n = 40, 46%), nausea (n = 40, 46%), and neutropenia (n = 37, 43%). Thirty-
eight (44%) patients experienced at least one drug-related grade ≥3 AE (Table 3);
the most common was neutropenia (n = 26, 30%). Drug-related grade ≥3 febrile
neutropenia was reported in two of 11 patients at the RP2D of 50 mg BID on the 7-
day schedule. Drug-related peripheral neuropathy was not a significant or frequent
finding, reported in only two patients who received doses above the MTD (one at 110
mg QD on the 7-day schedule; one at 60 mg BID on the 7-day schedule). CNS
disorders, including dizziness and somnolence, were reported in five of 11 patients
treated at the RP2D of 50 mg BID 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 due to AEs, including febrile neutropenia, neutropenia, and thrombocytopenia. One patient receiving 70 mg QD 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 prior 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 four (5%) on-study deaths within 30 days of the last dose of study drug, none of which were deemed to be drug-related.

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. Fig. 1 shows the mean plasma concentration–time profiles for patients receiving MLN8237 PIC 5–150 mg QD (panel A) and 50 or 60 mg BID (panel B) on the 7-day schedule. Based on 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 ($T_{\text{max}}$) of 2 h (range 1–6) post-dose. Mean steady-state $t_{1/2}$ following multiple dosing was approximately 23 h (coefficient of variation [CV]: 77%). Pharmacokinetic steady-state conditions were consistently achieved by approximately day 7 following daily oral administration based on similar mean $\text{AUC}_{0-\tau}$ 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 BID and QD
dosing, respectively, with corresponding mean accumulation ratios of 2.5 and 1.8 for
BID and QD dosing, respectively.

The steady-state exposure of MLN8237 increased approximately dose-
proportionally over the 5–150 mg QD dose range (Fig. 2A, \( n = 69 \)), supported by the
lack of dose-dependence of the steady-state apparent oral clearance (CL\(_{ss}/F\)) (Fig.
2B, \( n = 69 \)). At the RP2D of 50 mg BID on the 7-day schedule, the geometric mean
steady-state average concentration (C\(_{ss,avg}\)) 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 sub-study: ECT versus PIC**

Twenty-two patients received the ECT formulation: 10 mg BID and 20 mg BID
(each \( n = 1 \)) and 40 mg BID (\( n = 20 \)). 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\(_{max}\)
was 2.4 h for ECT and 2 h for PIC. The mean accumulation ratio of MLN8237
administered BID as ECT was approximately 2.8-fold, and the mean peak/trough
ratio was approximately 2.5, consistent with the values determined for BID dosing
with PIC.
Pharmacodynamics

Pre- and post-dose skin biopsies for assessment of the pharmacodynamic effects of MLN8237 on AAK inhibition were available from 58/59 patients in the dose-escalation portion of the study who received the PIC formulation. A post-treatment increase in skin MI, as demonstrated 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 pre-treatment 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 h after the day 1 dose and 6 h after the day 7 dose, including patients receiving the RP2D of 50 mg BID (Fig. 3).

Response and duration of treatment

Across all doses and schedules, patients received a median of two 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 PR lasting >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, two patients, one with skin cancer and one with NSCLC (bronchiolo-alveolar 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 demonstrated 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 performed 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 BID for 7 days in 21-day cycles. Higher cumulative doses per cycle and dosing schedules of longer duration (14- and 21-day) were not well tolerated.
At the RP2D, MLN8237 was generally well tolerated in this patient population. The most common AEs 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 AEs 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 (BID) doses on the 7-day schedule; this is likely to be related to the finding that BID 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 h. Over the dose range of 5–150 mg/day evaluated in this study, MLN8237 steady-state exposures increased approximately dose proportionally. Approximately 2.5-fold accumulation was observed with BID 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 BID (2.7 µM), exceeded the estimated efficacious steady-state plasma concentration of approximately 1 µM, which was consistently associated with antitumor activity in
This study also demonstrates 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 BID 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 h post-dosing 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 post-treatment biopsies. As ABK catalyzes the phosphorylation of pHistH3 (25), inhibition of ABK would have resulted in a post-treatment decrease in the frequency of pHistH3-positive cells (21, 33, 38).

It is possible that the post-treatment change in the skin MI would have been more pronounced later in the dosing schedule. Only a few of our patients had biopsies after seven days of dosing. A parallel phase 1 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 ((39) and Cervantes et al., manuscript submitted). Together, these data demonstrate that MLN8237 inhibits AAK in both skin and tumor at the tolerated doses, including the RP2D of 50 mg BID.

MLN8237 treatment was associated with antitumor activity in some heavily pre-treated 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, two patients achieved prolonged stable disease of 21.9 months and 24.5 months, respectively. The preliminary results reported in this phase 1 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 BID 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 1, 2, and 3 clinical studies in a variety of solid tumors and hematologic malignancies.
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References


### Tables

#### Table 1. Patient baseline demographics and disease characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N = 87</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years (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></td>
</tr>
<tr>
<td>Caucasian</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></td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>15 (17)</td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td>10 (11)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>10 (11)</td>
</tr>
<tr>
<td>Soft tissue sarcoma</td>
<td>8 (9)</td>
</tr>
<tr>
<td>Head and neck cancer</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Other*</td>
<td>38 (44)</td>
</tr>
<tr>
<td>ECOG performance status 0 / 1, n (%)</td>
<td>42 (48) / 45 (52)</td>
</tr>
<tr>
<td>Prior therapies, n (%)</td>
<td></td>
</tr>
<tr>
<td>Systemic therapy</td>
<td>85 (98)</td>
</tr>
<tr>
<td>Radiation</td>
<td>49 (56)</td>
</tr>
<tr>
<td>≥3 prior systemic therapies, n (%)</td>
<td>60 (69)</td>
</tr>
</tbody>
</table>

*Anal 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)
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><strong>7-day schedule</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mg QD</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10 mg QD</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>20 mg QD</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>40 mg QD</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>80 mg QD</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>110 mg QD</td>
<td>6</td>
<td>1</td>
<td>Somnolence, confusion, memory impairment (all grade 3)</td>
</tr>
<tr>
<td>150 mg QD</td>
<td>6</td>
<td>3</td>
<td>Somnolence (grade 2), confusion (grade 2), stomatitis (grade 3), asthenia (grade 3), neutropenia (grade 4)</td>
</tr>
<tr>
<td>50 mg BID</td>
<td>11</td>
<td>2</td>
<td>Febrile neutropenia (grade 4), thrombocytopenia (grade 4)</td>
</tr>
<tr>
<td>60 mg BID</td>
<td>6</td>
<td>2</td>
<td>Febrile neutropenia (grade 4), thrombocytopenia (grade 4)</td>
</tr>
<tr>
<td><strong>14-day schedule</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mg QD</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>40 mg BID</td>
<td>2</td>
<td>2</td>
<td>Diarrhea (grade 3), febrile neutropenia (grade 3), thrombocytopenia (grade 4)</td>
</tr>
<tr>
<td><strong>21-day schedule</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mg QD</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>50 mg QD</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>70 mg QD</td>
<td>7</td>
<td>1</td>
<td>Diarrhea (grade 3), thrombocytopenia (grade 4), ventricular dysfunction (grade 3), neutropenia (grade 4)</td>
</tr>
</tbody>
</table>
Table 3. Drug-related AEs of all grades (incidence >10%), and corresponding rates of grade ≥3 AEs (or those with an incidence >5%) observed in patients treated with MLN8237.

<table>
<thead>
<tr>
<th></th>
<th>All grades</th>
<th>Grade ≥3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with at least one drug-related AE, n (%)</td>
<td>85 (98)</td>
<td>38 (44)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>40 (46)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Nausea</td>
<td>40 (46)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>37 (43)</td>
<td>26 (30)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>36 (41)</td>
<td>0</td>
</tr>
<tr>
<td>Somnolence</td>
<td>31 (36)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>29 (33)</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Anemia</td>
<td>28 (32)</td>
<td>7 (8)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>23 (26)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>21 (24)</td>
<td>0</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>21 (24)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>10 (11)</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>7 (8)</td>
<td>7 (8)</td>
</tr>
</tbody>
</table>
Table and figure legends

Tables

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Supplementary Table S1. Summary of pharmacokinetic parameters for MLN8237 PIC formulation after multiple dose oral administration dosing on the 7-, 14-, and 21-day schedules.

Figures

Fig 1. Day 1 and day 7 concentration–time profiles for patients receiving 5, 10, 20, 40, 80, 110 or 150 mg QD (A), and 50 or 60 mg BID (B) MLN8237 PIC on the 7-day schedule.

Fig 2. Steady-state AUC$_{0-24h}$ (A) and apparent oral clearance (B) of MLN8237 versus total daily dose across QD and BID dosing cohorts following administration as a PIC formulation.

Fig 3. Mitotic index in patients with evaluable pre-dose and day 1 6-h (A), day 1 24-h (B), day 7 6-h (C), and day 21 6-h (D) post-dose skin biopsies.
Figure 2

A

BID (n = 27)  QD (n = 42)

AUC_{0-24hr} (nM/hr)

Total daily dose (mg/day)

B

BID (n = 27)  QD (n = 42)

CL/F (L/hr)

Total daily dose (mg/day)
Figure 3

A Change from baseline - day 1 6-h

B Change from baseline - day 1 24-h

C Change from baseline - day 7 6-h

D Change from baseline - day 21 6-h

Mitotic index (day x-h minus pre-dose)

Total daily dose (mg)
Phase 1 Study of Aurora A Kinase Inhibitor MLN8237 in Advanced Solid Tumors: Safety, Pharmacokinetics, Pharmacodynamics, and Bioavailability of Two Oral Formulations

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