Cancer Therapy: Clinical

Phase I Safety, Pharmacokinetic, and Pharmacodynamic Study of ENMD-2076, a Novel Angiogenic and Aurora Kinase Inhibitor, in Patients with Advanced Solid Tumors

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Abstract

Purpose: ENMD-2076 is a unique orally bioavailable Aurora kinase and VEGFR inhibitor. The purpose of this phase 1 study of ENMD-2076 was to determine the MTD, pharmacokinetic, and pharmacodynamic profiles and preliminary antitumor activity.

Experimental Design: Patients with refractory advanced solid malignancies were treated with ENMD-2076 orally with continuous once daily dosing. Doses from 60 to 200 mg/m² were evaluated using a standard 3 (to 4) + 3 design. Pharmacokinetic parameters were studied on days 1, 28, and 30 to 35 of cycle 1. Expanded MTD cohorts included patients with ovarian cancer, colorectal cancer, and refractory solid tumors.

Results: A total of 67 patients (46 F, 21 M; ages 30–76) entered the study. Dose levels of 60, 80, 120, 200, and 160 mg/m² were evaluated. Two patients experienced grade 3 hypertension at 200 mg/m², and additional grade 3 neutropenia events limited tolerability at this dose. An intermediate dose of 160 mg/m² was determined to be the MTD. The most common drug-related adverse events included hypertension, nausea/vomiting, and fatigue. The pharmacokinetics of ENMD-2076 were characterized by a rapid absorption phase (Tmax 3–7.8 hours), a t1/2 of 27.3 to 38.3 hours after a single dose, and dose proportional exposure. Decreased plasma sVEGFR2 was observed posttreatment. Two patients with platinum refractory/resistant ovarian cancer had RECIST partial responses.

Conclusions: ENMD-2076 was well tolerated, had a linear pharmacokinetic profile, and showed promising antitumor activity, particularly in ovarian cancer. The recommended phase 2 dose of ENMD-2076 is 160 mg/m² administered orally once daily with continuous dosing. Clin Cancer Res; 17(4); 849–60.

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Introduction

The Aurora kinases are a family of serine/threonine kinases integral to mitotic cell division. Aurora A plays a key regulatory role in centrosome function during mitosis including formation of the bipolar mitotic spindle (1, 2). Aurora B is a chromosomal passenger protein that plays a role in the attachment of chromosomes to the mitotic spindle and cytokinesis (3, 4). The Auroras are overexpressed in many solid tumors and have been implicated in tumorigenesis (5, 6). Multiple Aurora kinase inhibitors are currently in preclinical or clinical development (7).

Angiogenic kinases include VEGF receptors (VEGFRs), platelet-derived growth factor receptors (PDGFRs), and basic fibroblast growth factor receptors (bFGFRs). These receptors transduce signals from proangiogenic factors, promoting angiogenesis (8, 9), the process by which tumors recruit their vascular supply (10). Antiangiogenic agents can potentiate the effects of cytotoxic anticancer agents when used in combination. For example, the addition to chemotherapy of bevacizumab, a humanized monoclonal antibody directed at the proangiogenic factor VEGF, results in improved rate of response, progression free survival, and clinically meaningful increases in overall survival in advanced solid tumors such as colorectal cancer and non–small-cell lung cancer (11, 12).
ENMD-2076 was identified during the course of a focused synthetic medicinal chemistry effort directed toward discovery of novel inhibitors of Aurora kinases. However, in vitro assays defined the compound as an inhibitor of both Aurora and angiogenic kinases, targets of which include Aurora A (50% inhibitory concentration on recombinant kinase [IC50] 14 nM), Aurora B (IC50 350 nM), VEGFR2 (IC50 58 nM), FMS-like tyrosine kinase-3 (Flt-3; IC50 2 nM), stem cell factor receptor (c-Kit; IC50 120 nM), and FGFR1, 2 and 3 (IC50 93, 71 and 500 nM, respectively; ref. 13). These complementary antimitotic and antiangiogenic properties have translated to wide-ranging in vitro and in vivo antitumor activity against human cancer cell lines and xenografts, including models of breast cancer, colorectal cancer and hematologic malignancies (13–15). In vitro, antiproliferative effects are associated with G2/M cell cycle arrest and induction of apoptosis, most suggestive of an Aurora A phenotype (13, 16). At cellular concentrations below 500 nM in HCT116 colorectal cancer cells, these effects are accompanied by reductions in the auto-phosphorylation of Aurora A at Thr-288, consistent with selective Aurora A inhibition. At micromolar concentrations, in both HCT116 and multiple myeloma cells, reduced phosphorylation of Aurora A as well as histone H3 at Ser-10 are observed, the latter indicating concomitant inhibition of Aurora B (13, 15).

Dose-dependent inhibition of tumor growth has been observed in H929 human plasmaclastoma xenografts, with reduced expression of phospho-histone H3, Ki-67, CD34 in microvasculature and phospho-FGFR3, along with increased caspase-3 staining in tumors from mice treated with ENMD-2076 compared with vehicle (15). In the HT29 colorectal cancer xenograft model, exposure to 5 daily doses of ENMD-2076 reduced the number of mitotic cells staining positively for phosphorylated Aurora A (13). In longer-term experiments, ENMD-2076 induced initial tumor growth inhibition followed by regression that was accompanied by a decrease in 18FDG uptake assessed by positron emission tomography (PET) scanning, and associated with a marked reduction in proliferation, assessed by Ki-67. Treatment also caused reduced tumor vascular permeability and perfusion, measured by dynamic contrast-enhanced magnetic resonance imaging (14). Similarly, regressions in the MDA-MB-231 breast cancer xenograft model were associated with substantial reductions in tumor vascularization, assessed by CD31 staining (13).

In 28-day continuous oral dosing toxicology studies in rats and dogs, dose proportional increases in Cmax and AUC were seen. Gastrointestinal toxicities were observed in both species. In rats, liver function test abnormalities, bone marrow suppression, and adrenal cortical changes occurred. Also observed were dental discoloration and breakage, as well as abnormalities of the femoral growth plate attributable to disruption of the vascular invasion that occurs at the cartilage/bone interface during the process of endochondral ossification; the latter is a possible signature toxicity of pharmacologically induced inhibition of angiogenesis (17). Many of these changes were reversible after a 28-day recovery period. Additionally, no cardiovascular or respiratory system safety signals emerged in telemeterized dogs. Taken together, preclinical toxicology, pharmacology, and antitumor efficacy supported clinical assessment of ENMD-2076 in a first-in-human trial.

The principal objective of this study was to determine the maximum tolerated dose (MTD) of ENMD-2076 administered orally with continuous daily dosing in patients with advanced solid tumors. Secondary objectives included assessment of the toxicity profile, pharmacokinetics, pharmacodynamic effects on plasma soluble VEGFR2, and preliminary antitumor activity.

Patients and Methods

Eligibility criteria

Eligible patients had advanced or metastatic solid tumors refractory to or without standard therapy options, evaluable or measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST; ref. 18) and Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1. Patients also had to be age 18 years or older with adequate hematopoietic, hepatic, and kidney function. Exclusion criteria included: women who were pregnant or nursing; treatment with radiotherapy or chemotherapy within 2 weeks; major surgery within 3 weeks; known central nervous system metastasis requiring anticonvulsants or steroids; history of deep venous thrombosis or pulmonary embolism not receiving therapeutic anticoagulation; history of nethropic syndrome or + proteinuria; uncontrolled severe cardiac disease; any medical condition that would impair administration of an oral medication; or uncontrolled serious medical or psychiatric illness.
The protocol was approved by the institutional review boards of participating institutions, and written informed consent was obtained for all patients prior to performing study-related procedures in accordance with federal and institutional guidelines.

Drug administration and dose-escalation procedures
ENMD-2076 was supplied by EntreMed, Inc. and total daily dose was calculated based on body surface area. ENMD-2076 was supplied as capsules of various strengths and was orally administered to fasting patients on a once daily continuous dosing schedule. An exception to this was a 7-day observation period at the completion of cycle 1, prior to the initiation of cycle 2. ENMD-2076 was administered in the fasting state since a human food effect study has not been performed. The starting dose was 60 mg/m², calculated as one-tenth the dose that was lethal in 10% of rats (LD₁₀), the most sensitive species. This dose is also 3.3-fold below the no observed adverse effect level (NOAEL) in dogs.

For the dose-escalation portion of the study, patients were enrolled sequentially into escalating dosing cohorts using a standard 3(3 to 4) + 3 design. Initially, 5 dose levels were predetermined, but subsequently modified based on toxicity. Intrapatient dose escalation was not permitted. Dose-limiting toxicity (DLT) was defined as any grade ≥ 3 nonhematologic toxicity despite maximum supportive care (e.g., for nausea, diarrhea, or electrolyte abnormalities) or grade ≥ 4 hematologic toxicity occurring during the first cycle at least possibly related or of unknown relationship to ENMD-2076. If a single patient in any cohort of 3 to 4 patients developed DLT, the cohort was expanded to 6 patients. If 2 or more patients in any expanded cohort developed DLT, dose escalation was stopped and the cohort immediately below was further evaluated by expanding to include 6 patients. The MTD was defined as the highest dose level at which no more than 1 patient experienced DLT in a cohort of 6 patients. In the 200 mg/m² cohort, 2 patients were treated with 350 mg/m² for 2 days before being de-escalated to 200 mg/m² due to delayed toxicity occurring in the prior dosing cohort. Toxicology and clinical data from these patients were analyzed with the 200 mg/m² cohort, while day 1 PK analyzed with the 200 mg/m² cohort, while day 1 PK.

Clinical evaluation and safety assessment
Prior to study entry, patients underwent clinical history and physical examination, performance status assessment, vital signs, complete blood count (CBC), chemistries, coagulation parameters, urinalysis, serum pregnancy test, tumor markers, EKG, and baseline tumor measurements. While on study, patients underwent weekly evaluation including brief history and physical examination, vital signs, CBC and chemistries. Urinalysis was repeated each cycle and EKG repeated every other cycle. To address preclinical adrenal cortical pathology, cortisol levels were obtained on day 1 of each cycle, with a plan for an ACTH stimulation test if levels were below 80 nM/L. Based on known effects of other VEGFR inhibitors, TSH was obtained at baseline, at the conclusion of cycle 2, and subsequently if clinically indicated. Adverse events were classified/graded weekly according to the Common Terminology Criteria of Adverse Events, version 3.0. Response was assessed every 2 cycles and evaluated by modified RECIST (18).

Pharmacokinetic sampling and assay
In the dose escalation portion, blood samples were collected in sodium heparin tubes immediately prior to the first dose on cycle 1, day 1 and then at 0.5, 1, 2, 4, 6, 8, 10 to 12, and 24 hours following dosing. Samples were obtained on cycle 1, day 28 predose and at the same time points as on day 1. Additional samples were obtained on days 30, 31, and 35 (during the observation period) to better model terminal elimination rates. For cycle 2 and beyond, trough samples were obtained on day 15. In the MTD expansion arms, limited sampling occurred, including prior to the first dose on cycle 1, day 1, 24 hours following dosing, day 28, and day 15 for cycles 2 and beyond.

Plasma concentrations for ENMD-2076 and its active metabolite, ENMD-2060, were determined using a validated liquid chromatography tandem mass spectrometric method (LC/MS/MS) using ion transition parameters optimized for each analyte. A manuscript is in preparation with detailed procedures. The lower limit of quantification for both ENMD-2076 and ENMD-2060 was 1 ng/mL. Pharmacokinetic parameters were calculated from plasma concentration-time data with standard noncompartmental methods using Excel.

Pharmacodynamic analysis
Blood samples for plasma soluble VEGFR2 (sVEGFR2) were collected immediately prior to dosing on cycle 1, day 1 and on cycle 1, day 28. During subsequent cycles, a single blood sample was obtained immediately prior to dosing on day 15. Plasma samples were diluted 1:5 and concentrations determined by ELISA, per the manufacturer’s instructions (R&D Systems). ELISA plates were read at 450 nm on a Synergy 2 plate reader (Biotek).

Skin biopsies were performed in a subset of patients enrolled in the MTD expansion arms. Formalin-fixed, paraffin-embedded samples were analyzed by immunohistochemistry using an anti-phospho-histone H3 (pHH3) antibody (Cell Signaling Technology) as a marker of Aurora inhibition (19).

Statistical methods
Descriptive statistics were used for baseline characteristics, safety assessments, pharmacokinetic variables (including $C_{\text{max}}$, $T_{\text{max}}$, AUC₀⁻⁻¹, and $t_{1/2}$) and exploratory assessments, including time to progressive disease,
tumor response and pH3 immunohistochemistry in keratinocytes. Measurements of sVEGFR2 before and during ENMD-2076 were subjected to the unpaired Student's t-test. Linear regression was used to evaluate the relationship of pharmacokinetic variables with dose and patient age, weight, estimated creatinine clearance and albumin concentration. Correlation of percent decrease of plasma sVEGFR2 and reduction of tumor measurements was assessed by Pearson's correlation coefficient. P values < 0.05 were considered statistically significant. Statistical analyses were performed using GraphPad Prism software.

Results

Patient demographics and treatment

Sixty-seven patients, 29 in the dose-escalation portion and 38 in the MTD expansion, were enrolled from April 2008 to October 2009 (Table 1). A total of 257 cycles were administered. The median number of cycles per patient was 3 (range, 1–24). Reasons for study discontinuation were progressive disease in 41 patients (61%), an adverse event in 6 (9%), and other reasons in 11 (17%). At the time of data analysis, 9 patients (13%) continue on treatment.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Dose escalation (N = 29)</th>
<th>Expansion at MTD (N = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
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<tr>
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<td>41</td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
<td>59</td>
</tr>
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<tr>
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<td></td>
</tr>
<tr>
<td>Neuroendocrine tumor</td>
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<tr>
<td>Other</td>
<td>4*</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ECOG, Eastern Cooperative Oncology Group; VEGF, vascular endothelial growth factor; TKI, tyrosine kinase inhibitor.

*One patient each with bladder cancer, melanoma, thyroid cancer, and urachal cancer.
†One patient each with bladder cancer, melanoma, adenoid cystic carcinoma, alveolar soft parts sarcoma, endometrial cancer, gastric cancer, hepatocellular carcinoma, and mesothelioma.
Dose escalation and determination of MTD

Doses from 60 to 200 mg/m^2 were evaluated. The dose-escalation scheme, median number of cycles, frequencies of dose delay and dose reductions, and DLTs in cycle 1 by dosing cohort are summarized in Table 2. One patient experienced a DLT in the first cohort (60 mg/m^2) with grade 4 hypertension and grade 3 pain in the setting of grade 3 cholecystitis. The patient recovered and was treated with a dose reduction to 30 mg/m^2 for 5 cycles before coming off study for disease progression. This cohort was expanded to include 7 patients and no further DLTs were observed. Patients were subsequently enrolled to dosing cohorts 2 (80 mg/m^2) and 3 (120 mg/m^2) with no DLTs observed. Among 4 patients initially treated in the fourth dosing cohort (200 mg/m^2), no dose-limiting toxicities were observed, prompting enrollment to the fifth dosing cohort at 350 mg/m^2. However, at the time the first 2 patients initiated dosing at 350 mg/m^2, grade 3 neutropenia beyond cycle 1 was documented in 1 patient at the 200 mg/m^2 dose level. Study drug was held and eventually resumed at 120 mg/m^2 once the absolute neutrophil count improved to grade 2. However, it was felt that additional patients should be evaluated at 200 mg/m^2, so that the 2 patients in the fifth dosing cohort received 350 mg/m^2 for only 2 days and were subsequently de-escalated to 200 mg/m^2. One additional patient was enrolled at 200 mg/m^2 to comprise an additional cohort of 3 patients at this dose level. Among this latter group, 2 patients experienced grade 3 hypertension meeting DLT criteria. Furthermore, among the entire group treated at 200 mg/m^2, 3 patients developed grade 3 neutropenia requiring dose delay and reduction, although not meeting DLT criteria. Therefore, the 200 mg/m^2 dose level was not considered tolerable and an intermediate dose level of 160 mg/m^2 was evaluated. At 160 mg/m^2, 1 of 8 patients experienced DLT with grade 3 fatigue, dehydration, and hyponatremia. These events occurred in the setting of rapid disease progression ultimately leading to death. The 160 mg/m^2 dose level was chosen as the MTD, at which the expansion arms were enrolled.

Safety

The most frequently observed drug-related adverse events seen in all courses were hypertension, nausea and/or vomiting, fatigue, and diarrhea (Table 3; Supplementary Tables S1 and S2). Nausea and/or vomiting were observed in 10/29 patients (38%) during dose escalation and 16/38 (42%) in the expanded 160 mg/m^2 cohort. Nausea was a reason for dose reduction in only 1 patient. Grade 3 and 4 drug-related events included hypertension, fatigue, diarrhea, dyspnea, dehydration, neutropenia, hypophosphatemia, and elevated AST/ALT. Toxicity events leading to dose reduction in 2 or more patients included fatigue, hypertension, neutropenia, dyspnea, abdominal pain, and diarrhea. Fatigue was more commonly seen at higher dosing levels including 12/38 (32%) of patients in the expanded 160 mg/m^2 cohort, reaching grade 3 to 4 severity in 2 of these patients and necessitating dose reduction in 5 patients. Fatigue was not explained by hypothyroidism, which occurred in only 2 patients, both in the expanded 160 mg/m^2 cohort. Of note, despite preclinical concerns, adrenal insufficiency was not observed.

Hypertension was seen in 55% of total patients, occurred at all dose levels and reached grade 3/4 severity in 21% (Table 3). In most cases, hypertension was easily controlled with addition of 1 antihypertensive agent (71%), most commonly a dihydropyridine calcium channel blocker or an angiotensin-converting enzyme inhibitor. Hypertension resulted in dose delay or reduction in 16% of patients, but only resulted in study discontinuation for 1 patient. The median time to onset of hypertension following initial dosing was 14 days (range 2–60 days). A prior history of hypertension was noted in 46% of these patients. There was

### Table 2. Number of patients with dose delays, reductions and DLTs per dose level

<table>
<thead>
<tr>
<th>Cohort no.</th>
<th>ENMD-2076 dose level (mg/m²)</th>
<th>No. of patients</th>
<th>No. of cycles</th>
<th>No. of patients with dose delays</th>
<th>No. patients with dose reductions</th>
<th>No. of patients with DLT (cycle 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>7</td>
<td>2</td>
<td>1-24*</td>
<td>2 (29%)</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>4</td>
<td>4.5</td>
<td>3-6</td>
<td>3 (75%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>2</td>
<td>2</td>
<td>2-3</td>
<td>1 (33%)</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>8</td>
<td>3</td>
<td>1-6</td>
<td>7 (100%)</td>
<td>3 (43%)</td>
</tr>
<tr>
<td>5</td>
<td>160</td>
<td>24</td>
<td>8</td>
<td>1-11*</td>
<td>3 (38%)</td>
<td>1 (13%)</td>
</tr>
<tr>
<td>Expansion</td>
<td>160</td>
<td>38</td>
<td>3</td>
<td>1-8*</td>
<td>24 (63%)</td>
<td>14 (37%)</td>
</tr>
</tbody>
</table>

Abbreviations: DLT, dose-limiting toxicity.

*Includes patients still receiving study drug.

†Grade 4 hypertension and grade 3 pain in the setting of grade 3 cholecystitis in 1 patient.

‡Two patients with grade 3 hypertension and 3 patients with neutropenia requiring dose delay not meeting DLT criteria, but dose level was not felt to be feasible.

§Grade 3 dehydration, fatigue, and hyponatremia in 1 patient.

The most frequently observed drug-related adverse events seen in all courses were hypertension, nausea and/or vomiting, fatigue, and diarrhea. Nausea and/or vomiting were observed in 10/29 patients (38%) during dose escalation and 16/38 (42%) in the expanded 160 mg/m² cohort. Nausea was a reason for dose reduction in only 1 patient. Grade 3 and 4 drug-related events included hypertension, fatigue, diarrhea, dyspnea, dehydration, neutropenia, hypophosphatemia, and elevated AST/ALT. Toxicity events leading to dose reduction in 2 or more patients included fatigue, hypertension, neutropenia, dyspnea, abdominal pain, and diarrhea. Fatigue was more commonly seen at higher dosing levels including 12/38 (32%) of patients in the expanded 160 mg/m² cohort, reaching grade 3 to 4 severity in 2 of these patients and necessitating dose reduction in 5 patients. Fatigue was not explained by hypothyroidism, which occurred in only 2 patients, both in the expanded 160 mg/m² cohort. Of note, despite preclinical concerns, adrenal insufficiency was not observed.
no correlation between the development of hypertension and clinical benefit (data not shown).

Neutropenia was first seen at 200 mg/m² during dose escalation in 4 of 7 patients (57%); 3 of 7 (43%) had grade 3 neutropenia requiring dose delay and reduction (Table 3). One episode of grade 4 neutropenia was seen in this cohort during cycle 3, and so did not constitute a DLT. In the expanded 160 mg/m² cohort, 3 of 38 patients (8%) developed grade 1/2 neutropenia, and 2 of 38 (5%) developed grade 3/4 neutropenia (Supplementary Table S2). The median time to onset of neutropenia was 36 days (range 19–181 days), and the median time to recovery was 12 days (range 6–19 days). With dose reduction to 120 mg/m² in patients with grade 3 neutropenia at higher doses, further instances of neutropenia requiring dose modification were not encountered. There were no cases of neutropenic fever and no patient required growth factor support.

Oral mucosal-related complaints were common with ENMD-2076 treatment. In patients treated at all dose levels, 11/67 (16%) experienced glossodynia, 10/67 (15%) experienced mucositis, and 7/67 experienced (10%) dysgeusia. These AEs were more common at higher dosing levels, and symptoms were often exacerbated by carbonated beverages or spicy foods. Four patients treated in the expanded 160 mg/m² cohort experienced tooth discoloration.

Dose delays occurred in 24/38 (63%) of patients in the MTD expansion. In 4 patients, the reason for delay was an intercurrent illness not due to ENMD-2076. The most common reasons for ENMD-2076-related dose delays included fatigue (n = 6) and hypertension (n = 5). The frequency of dose-reductions by dosing cohort is shown in Table 2 and individual events are listed in Supplementary Table S3. In the MTD expansion, 14/38 (37%) required dose reduction. The reasons for dose reduction in this group included: fatigue (n = 5), hypertension (n = 2), diarrhea (n = 2), neutropenia (n = 2), dyspnea (n = 2), abdominal pain (n = 1), hyponatremia (n = 1), polycythemia (n = 1), and mucositis (n = 1). Dose reduction was based on multiple adverse events in 3 patients; only 1 patient was dose-reduced during the first cycle.

In the MTD expansion, 12/38 (32%) patients experienced 16 adverse events meeting DLT criteria. The majority of these (n = 7) were due to hypertension of grade 3 (n = 6) or grade 4 (n = 1) severity. Other events included grade 3 fatigue (n = 2), elevated AST/ALT (n = 2), elevated alkaline phosphatase (n = 1), hypophosphatemia (n = 1), hyponatremia (n = 1), congestive heart failure (n = 1), and dyspnea (n = 1).

### Pharmacokinetic studies

Pharmacokinetic parameters were determined for ENMD-2076 and ENMD-2060, an active metabolite (Table 4), and plasma concentrations plotted by dosing cohort (Fig. 1A–C). The absorption kinetics of ENMD-2076

| Table 3. Treatment-related hematologic and nonhematologic adverse events: worst grade per patient in all cycles in at least 10% of all patients and laboratory changes in at least 5% of patients |
|-----------------|-----------------|-----------------|
| Adverse event*  | Any grade | Grade 3 | Grade 4 |
| Patients reporting related adverse events | 61 | 91 | 19 | 28 | 6 | 9 |
| Hypertension | 37 | 55 | 12 | 18 | 2 | 3 |
| Nausea/vomiting | 26 | 39 | 0 | 0 | 0 |
| Fatigue | 21 | 31 | 2 | 3 | 0 | 0 |
| Diarrhea | 17 | 25 | 2 | 3 | 0 | 0 |
| Anorexia | 16 | 24 | 0 | 0 | 0 |
| Dehydration | 12 | 18 | 1 | 1 | 0 | 0 |
| Glossodynia | 11 | 16 | 0 | 0 | 0 |
| Constipation | 10 | 15 | 0 | 0 | 0 |
| Mucositis | 10 | 15 | 0 | 0 | 0 |
| Dyspnea | 8 | 12 | 3 | 4 | 0 | 0 |
| Dysgeusia | 7 | 10 | 0 | 0 | 0 |
| Laboratory changes occurring in ≥5% of patients | 9 | 13 | 3 | 4 | 2 | 3 |
| Neutropenia | 11 | 16 | 0 | 0 | 0 |
| Proteinuria | 7 | 10 | 1 | 1 | 0 | 0 |
| Hypophosphatemia | 7 | 10 | 1 | 1 | 2 | 3 |
| Elevated AST, ALT, or Alk Phos | 4 | 6 | 0 | 0 | 0 |
| Anemia | 4 | 6 | 0 | 0 | 0 |

Abbreviations: ALT, alanine amino-transferase; AST, aspartate amino-transferase.

*Graded per National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.
were rapid after single and multiple doses with time to maximum observed plasma concentration (T\text{max}) ranging from 3.0 to 7.8 hours for day 1 and 2.7 to 4.7 for day 28 over all dose levels. The mean terminal phase elimination half-life (t\text{1/2}) after a single dose is estimated to range from 27.3 to 38.3 hours and from 44.9 to 53.1 hours after multiple doses. Using noncompartmental modeling, the maximum plasma concentration (C\text{max}) and area under the plasma concentration-time curve (AUC\text{0-inf}) were dose proportional after single and multiple doses (Fig. 1D and E). With continuous daily dosing during cycle 1, accumulation of ENMD-2076 was observed with an average factor of 3.4 (±1.1) across all doses (C\text{max} day 28/C\text{max} day 1). However, additional accumulation was not seen based on analysis of subsequent trough levels in a subset of patients who remained on study for greater than 2 cycles (Supplementary Fig. S1).

The active metabolite, ENMD-2060, reached a maximum plasma concentration (C\text{max}) between 6.0 to 19.4 hours on day 1 and 2.7 to 11.4 hours on day 28. ENMD-2060 had a longer t\text{1/2} than ENMD-2076, which ranged from 89.9 to 139 hours, and by linear regression, there was a significant inverse correlation between t\text{1/2} and dose cohort (P = 0.0171). There was no statistically significant dose proportionality in either AUC\text{0-inf} or C\text{max} for ENMD-2060 (Fig. 1F).

### Correlation of patient characteristics with ENMD-2076 and ENMD-2060 pharmacokinetic variables

Patient age, weight, estimated creatinine clearance, and albumin concentration were independently analyzed for correlation to pharmacokinetic variables of ENMD-2076 on days 1 and 28 and of ENMD-2060 on day 28. Patient weight correlated with ENMD-2076 AUC\text{0-inf}/fixed dose for day 1 (P = 0.0095), as well as C\text{max}/fixed dose (P = 0.0493) and Vd/F (P = 0.0495) for day 28 (Supplementary Fig. S2A–C). Patient weight also correlated with ENMD-2060 fixed dose corrected C\text{max} (P = 0.0093) for day 28 (Supplementary Fig. S2D). No other correlations between patient characteristics and pharmacokinetic parameters were observed.

### Pharmacodynamic analysis: plasma sVEGFR2

The effect of ENMD-2076 on plasma sVEGFR2 was determined after 28 days of continuous dosing. The mean plasma sVEGFR2 decreased from 8,419 pg/mL (95% CI...
7,716, 9,122) at cycle 1, day 1 to 5,600 pg/mL (95% CI 5,086, 6,113) at cycle 1, day 28 (P < 0.001; Fig. 2A). A decrease in sVEGFR2 at day 28 compared to baseline was seen in all dosing cohorts (Fig. 2B), and persisted through multiple treatment cycles (data not shown). There was no association between percent decrease in sVEGFR2 and maximum decrease in tumor measurements by modified RECIST by Pearson’s Correlation (r² = 0.027, P = 0.28).
Histone H3 phosphorylation in keratinocytes

Pretreatment and on-treatment (cycle 1, day 28) skin biopsies were obtained from 9 patients treated at the MTD and analyzed by immunohistochemistry to determine if there was a consistent pattern of increased or decreased HH3 phosphorylation on treatment, suggestive of Aurora A or Aurora B inhibition, respectively (19). Consistent with results of previous studies, the number of pHH3-positive cells detected was low, ranging from 0 to 3/section (20, 21). No clear trend emerged. Among the 9 paired biopsies, the number of pHH3-positive cells decreased posttreatment in 3 samples, remained the same in 4 samples and increased in 2 samples (Supplementary Table S4 and Supplementary Fig. S3).

Antitumor activity

Fifty-eight out of 67 patients were evaluable for tumor response. Of these, 2 patients (3%) had partial responses (PR; 1 confirmed), 49 had stable disease (SD; 85%), and 7 had progressive disease (PD; 12%) as their best response (Fig. 3). Both patients with PR have ovarian cancer. The first, considered platinum-resistant, was treated at the initial dosing cohort (60 mg/m²) and remains on study after 24 months. The second, considered platinum-refractory, was treated at 160 mg/m², and remains on study after 6 months. Twenty patients (30%) had decreases in tumor measurements from baseline that did not qualify as PR. Multiple patients who experienced PR or SD ≥ 12 weeks had prior exposure to anti-VEGF therapy (Supplementary Table S5). Patients who experienced clinical benefit for greater than 6 months included 5 patients with ovarian cancer, as well as patients with hepatocellular carcinoma, neuroendocrine carcinoma (including large cell and insulinoma), melanoma, colorectal carcinoma, renal cell carcinoma, triple-negative breast cancer, and alveolar soft part sarcoma. Of the 20 ovarian cancer patients enrolled at all dose levels, 18 were platinum-resistant or refractory. Twelve (60%) experienced PR or SD ≥ 12 weeks and 9 (45%) had a 50% decrease in CA125 (Supplementary Fig. S4). Of the 19 colorectal cancer patients enrolled, 5 (26%) had SD ≥ 12 weeks, including patients with Kras-mutant tumors. There was no correlation between dose-level and responses.

Discussion

ENMD-2076 is a novel multitargeted kinase inhibitor that combines antimitotic and antiangiogenic activity through inhibition of both Aurora and angiogenic kinases. Pharmacokinetics of ENMD-2076 demonstrated dose-proportionality of both Cmax and AUC0-inf. ENMD-2076 was well tolerated based on the toxicity profile and prolonged duration of treatment in many patients. The most common adverse events, including hypertension, fatigue, nausea, and diarrhea, were consistent with those reported with other antiangiogenic agents (22–29). Myelosuppression was seen at higher dose levels as has occurred with other Aurora kinase inhibitors (20, 21, 30, 31) as well as multitargeted kinase inhibitors such as sunitinib and sorafenib, whose targets, like ENMD-2076, include c-Kit and Flt3 (32, 33).

At the MTD of 160 mg/m², 33% of patients required dose reduction due to toxicity, similar to rates of dose modification required with other multitargeted kinase inhibitors (22, 24). For example, among 375 renal cell carcinoma patients treated at the recommended phase 2 dose of sunitinib in the comparison with interferon-alfa, 38% and 32% experienced at least 1 treatment-related dose reduction.
delay or reduction, respectively. Additionally, the rates of grade ≥ 3 fatigue (7%), hypertension (8%), and neutropenia (11%; refs. 22, 23) were comparable to the rate of such events among the 46 patients treated with ENMD-2076 at 160 mg/m², which were 7% (3/46), 24% (11/46), and 4% (2/46), respectively. The 160 mg/m² dose is tolerable based on protocol definitions during the first cycle and has a manageable side effect profile thereafter, with hypertension readily controlled. In future phase 2 studies, it may be informative to investigate 2 dose levels of ENMD-2076, the RP2D and a dose level below, to further characterize the toxicity profile and a possible dose-effect with treatment. Considering the long half-life and late appearance of toxicities in some patients, intermittent schedules (e.g. 3 weeks on/1 week off) could also be evaluated.

A decrease in plasma sVEGFR2 was seen with ENMD-2076 treatment at all dose levels and persisted through many treatment courses. This is congruent with effects seen with other VEGFR2 inhibitors (32), and demonstrates in vivo VEGFR2 inhibition at doses of ENMD-2076 below the MTD. In contrast, pharmacodynamics of Aurora kinase inhibition were not established among a small set of skin biopsies analyzed at MTD. Of note, this dose level produced only mild neutropenia, so effects of ENMD-2076 on Aurora activity may have been modest. In preclinical models, ENMD-2076 induces G2/M arrest consistent with Aurora A inhibition (13, 16), and increases in mitotic index, evidenced by increased pH3 staining, were seen in 2 samples. However, exposures achieved suggest that combined Aurora A/B inhibition was possible, which may account for reduced pH3 after treatment in some samples (19). Other explanations for the lack of an anticipated increase in pH3 following treatment include the small sample size, small numbers of pH3 positive cells per sample, and unknown skin penetration of free ENMD-2076. Further work to address the effects of ENMD-2076 on Aurora kinase activity in tumor cells is ongoing in a phase 1 study in patients with relapsed acute myelogenous leukemia (34).

In this heavily pretreated population, including 67% having received prior antiangiogenic agents, prolonged clinical benefit was seen in patients with a variety of solid tumors, notably in ovarian cancer. Two patients with platinum-resistant disease achieved PR (1 confirmed). Several patients remained on ENMD-2076 at least 6 months, with multiple CA125 reductions observed.

Figure 3. Waterfall plot of the change in the sum of the longest diameters of target lesions observed at the time of best response. Not shown, n = 9, due to unavailable tumor measurements. Dose cohorts are shown in parentheses. White bars and/or *s represent patients who remained on ENMD-2076 at least 6 months.
A correlation between the development of hypertension and clinical benefit has been reported with a number of antiangiogenic agents, including bevacizumab, sunitinib, and axitinib (35–37). We did not observe this association with ENMD-2076. There was also no correlation between the magnitude of decrease in plasma sVEGFR2 and clinical outcome. Furthermore, prior exposure to antiangiogenic therapy did not preclude clinical benefit. This may indicate a lack of cross-resistance between ENMD-2076 and other antiangiogenic agents, or the additional impact of concomitant Aurora kinase and/or FGFR inhibition.

In summary, daily oral ENMD-2076 was well tolerated in solid tumor patients to at least 24 months with promising preliminary anticancer activity. These data support future clinical trials evaluating this agent, both alone and in combination. A single-agent phase 2 study of ENMD-2076 in ovarian cancer is underway.

Disclosure of Potential Conflicts of Interest


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