A Phase I Study of ENMD-2076 in Patients with Relapsed or Refractory Acute Myeloid Leukemia (AML)

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UPDATED ABSTRACT

BACKGROUND. ENMD-2076 is a novel, orally-active molecule that inhibits Aurora A kinase and FLT3, as well as multiple receptor tyrosine kinases that drive tumor vascularization, including VEGFR2 (KDR), PDGR and FGFR. A phase I study was conducted to determine the maximum tolerated dose (MTD) and toxicities of ENMD-2076 in patients with refractory hematological malignancies.

METHODS. Patients (cohorts of 6 evaluable patients per dose level) received escalating doses of ENMD-2076 administered orally daily [225 mg (n=7), 375 mg (n=6), or 325 mg (n=7)]. Plasma levels of ENMD-2076 were measured on Days 1, 8, & 29 of cycle 1 and at end of study. Peripheral blood and/or bone marrow were obtained at baseline for ex vivo drug sensitivity testing, and on Days 8 & 29 of cycle 1 for pharmacodynamic (PD) monitoring to measure the effects of ENMD-2076 on cell signaling pathways. This flow cytometry-based assay uses combined labeling for pERK, pAkt, pSTAT5, and pS6 as the readout, and tests the effects of acute stimulation with the ligands SCF and FLT3L in the presence or absence of pathway inhibitors, including ENMD-2076.

RESULTS. Twenty patients have been treated to date (all having a diagnosis of AML). Median age is 70 years (range, 43-82 years). Median ECOG status 1 (range, 0 to 2). Eighteen patients (90%) had received prior therapy (median, 2 regimens; range, 0 to 6 regimens); 1 had received a prior allogeneic stem cell transplant. A total of 42 cycles have been administered, with a median of 1 cycle (range, 0 to 5 cycles); 6 patients (30%) have received 2 or more cycles of therapy. The most common nonhematological toxicities of any grade, regardless of association with drug, were fatigue, diarrhea, dizziness, cough, constipation, nausea, and bony pain. Two of 6 patients treated at the 375 mg/day dose level developed dose-limiting toxicities (DLT), consisting of grade 3 fatigue. Therefore, the dose was decreased to 325 mg/day; one patient on this dose level developed a DLT with grade 3 fatigue. No patient experienced grade 4 toxicities or death from ENMD-2076. Of the 15 evaluable patients, one patient achieved a CRi (transfusion-independent with platelets <100 x 10^9/L), two a morphologic leukemia-free state (MLFS) with a major HI-P and/or HI-E, and 3 other patients had an 11%, 14%, and 65% reduction in marrow blast count, respectively. Five of these patients remain on study for 0.9 to 5.1+ months. Ex vivo study showed pre-incubation with ENMD-2076 concentrations in the range of 0.5-2μM suppressed growth factor stimulation in the blast cells of most patients tested to date. Decreases in the ability to stimulate ERK, Akt, STAT5, and S6 were seen in the Day 8 & 29 samples, including a striking inhibition of cell signaling in one patient who achieved a CRi.

CONCLUSIONS. Single agent ENMD-2076 has activity in a heavily pretreated group of AML patients that may correlate with inhibition of ERK, Akt, STAT5, and S6 activity. Enrollment, as well as PK and PD monitoring of this study, is ongoing.

BACKGROUND

- ENMD-2076 is a selective inhibitor of the Aurora A kinase isoform in comparison to Aurora B (IC_{50} 14 nM *vs* 350 nM)
- ENMD-2076 inhibits the activity of multiple kinases, including Flt3, c-Kit, & CSF-1R, which are involved in hematological malignancies
- The compound also has potent activity against receptor tyrosine kinases that are involved in angiogenesis and lymphangiogenesis, such as VEGFR2 (KDR), PDGFR, and FGFR

OBJECTIVES

- To define MTD and DLT of oral daily ENMD-2076 in patients with relapsed or refractory hematological malignancies
- To determine safety & toxicity of repeated oral dosing of ENMD-2076
- To assess for anti-cancer effects of ENMD-2076
- To determine the PK profiles & PD effects of ENMD-2076 on signaling pathways, and on Aurora A kinase and cell cycle in the cancer cells

METHODS

STUDY DESIGN

- Phase 1 ENMD-2076 dose escalating trial starting at a dose of 225 mg orally daily in 28-day cycles
- Cohorts of 6 evaluable patients per dose level
- Duration of therapy: Indefinite unless disease progression, intercurrent illness/medical condition, unacceptable toxicity, or patient request

ELIGIBILTY CRITERIA

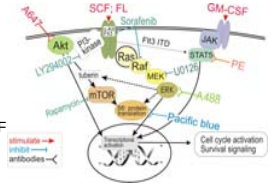
- Relapsed or refractory AML, acute lymphocytic leukemia (ALL), or chronic myeloid leukemia in blast crisis (CML-BC), chronic myelomonocytic leukemia (CMML), chronic lymphocytic leukemia (CLL), agnogenic myeloid metaplasia (AMM), lymphoma or myeloma
- Patients with untreated AML who refuse induction chemotherapy or are not candidates for aggressive management are also eligible

PHARMACODYNAMIC MONITORING

Complex flow cytometry protocol developed to study multiple potential drug targets:

1) assess baseline signaling in leukemic blast cells;

2) ex vivo drug effects on c-Kit activation. Cells were stimulated with the ligands SCF and FLT3L in the presence or absence of pathway inhibitors [i.e. U0126 (Mek inhibitor), LY294002 (PI3 kinase inhibitor), rapamycin (mTOR inhibitor), and sorafenib (FLT3 and Raf kinase inhibitor) and ENMD-2076].



3) monitor pharmacodynamic effects during treatment.

Peripheral blood or bone marrow were fixed with 4% formaldehyde, RBC lysed with 0.1% Triton X-100, and epitope unmasked with 80%methanol. Five color staining (pERK-Alexa488, pSTAT5-PE, pAkt-Alexa647, pS6-Pacific Blue, CD34/117-PC7) was done in a single tube, and a three laser instrument (Gallios; Beckman-Coulter) used for data acquisition. Blast cells were gated on the side scatter/CD34/117+ population. Analysis was done with FCS Express (De Novo software).

RESULTS

Table 1. Patient characteristics (n=20)

Characteristic	All (n=20)
Age, y (median)	**70 (43-82)**
Gender	**5 F : 15 M**
ECOG status (median)	**1 (0-2)**
Acute myeloid leukemia	
De novo	14 (70%)
Therapy/Secondary	6 (30%)
Disease duration, mo (median)	**11.2 (1.1-76.8)**
Flt3-ITD mutation status	
Negative	10 (50%)
Positive	2 (10%)
Unknown	8 (40%)
No. prior therapies (median)	**2 (0-6)**
None	2 (10%)
1 to 6 regimens*	18 (90%)

* allogeneic SCT (n=1)

Table 2. Most Common Non-Hematological Toxicities, regardless of attribution, for Cycle 1 (n=15)

Adverse Effect	Grade 1/2 No. (%)	Grade 3/4 No. (%)	Total No. (%)
Fatigue	6 (40)	3 (20)	9 (60)
Diarrhea	5 (33)	0	5 (33)
Cough	4 (27)	0	4 (27)
Constipation	3 (20)	1 (7)	4 (27)
Dizziness	4 (27)	0	4 (27)
Nausea	4 (27)	0	4 (27)
Bony pain	3 (20)	1(7)	4 (27)
Bruising	3 (20)	0	3 (20)
Confusion	1 (7)	2 (13)	3 (20)
Dry mouth	3 (20)	0	3 (20)
Dysphonia	3 (20)	0	3 (20)
Epistaxis	2 (13)	1 (7)	3 (20)
Hypertension	3 (20)	0	3 (20)
Hypomagnesemia	3 (20)	0	3 (20)
Shortness of breath	3 (20)	0	3 (20)
Weakness	3 (20)	0	3 (20)

- 2 of 6 patients at dose level 375 mg/day developed DLT (grade 3 fatigue); therefore, the dose was reduced to 325 mg/day
- 1 of 7 patients currently being treated at dose level 325 mg/day developed DLT (grade 3 fatigue)
- No other drug-related DLTs were observed

Table 3. Overall responses by IWG criteria (2003) (n=15)

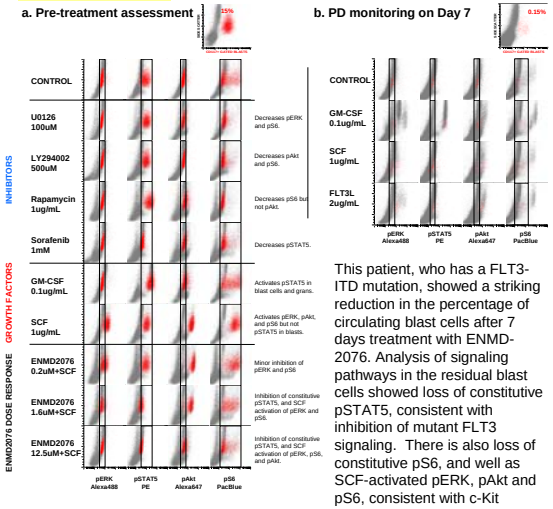
	All (n=15)
$CR_{incomplete}$	1
Morphological LFS with major HI-P &/or HI-E	2
Overall Response	**3 (20%)**

- 3 other patients (patients #119, 112, & 120) had an 11%, 14%, and 65% reduction in marrow blast count, respectively
- 5 of these patients remain on study for 0.9 to 5.1+ months

STUDY PATIENT 106

a. Pre-treatment assessment

b. PD monitoring on Day 7



This patient, who has a FLT3-ITD mutation, showed a striking reduction in the percentage of circulating blast cells after 7 days treatment with ENMD-2076. Analysis of signaling pathways in the residual blast cells showed loss of constitutive pSTAT5, consistent with inhibition of mutant FLT3 signaling. There is also loss of constitutive pS6, and well as SCF-activated pERK, pAkt and pS6, consistent with c-Kit inhibition during treatment with ENMD-2076.

Summary of pharmacodynamic monitoring to date

We have analyzed samples from 15 study patients, and this work is ongoing. Results using this highly complex flow cytometry assay show considerable inter-patient heterogeneity in baseline signaling activity, as well as the response to study drug. A second patient, #120, has a signaling profile very similar to #106, and early follow up indicates that this patient has also had a major response, suggesting that this pattern might be a useful predictive biomarker for ENMD-2076 sensitivity.

CONCLUSIONS

- Single agent ENMD-2076 has activity in patients with relapsed or refractory AML
- Enrollment as well as PK and PD monitoring of this study, is ongoing
- Additional flow cytometry monitoring of effects of ENMD-2076 on Aurora kinases and the cell cycle is being performed [using combined staining for cyclin A2, pHistone H3 (Ser 10), and DNA content]