A Phase I Study of ENMD-2076 in Patients with Relapsed or Refractory Leukemia

KWL Yee, HWT Chen, J Brandwein, S Chow, D Sanfelice, D Hedley, V Gupta, A Schimmer, CF Sidor, G Fletcher, J Arnott, M Bray, MD Minden, A Schuh

Princess Margaret Hospital, Toronto, ON, Canada; EntreMed, Inc., Durham,NC, USA; EntreMed, Inc., Rockville, MD, USA; EntreMed, Inc., Toronto, ON, Canada

UPDATED ABSTRACT

BACKGROUND. ENMD-2076 is a novel, orally-active molecule that inhibits Aurora A kinase, c-Kit, 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), 325 mg (n=9), or 275 mg (n=5)]. 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. The latter flow cytometry-based assay used combined pERK, pAkt, pSTAT5, and pS6 labeling, and tested the effects of acute stimulation with SCF and FLT3 ligands in the presence or absence of pathway inhibitors, including ENMD-2076.

RESULTS. Twenty-seven patients were treated (26 AML; 1 CMML-2). Median age of 69 years (range, 43-84 years). Median ECOG status 1 (range, 0 to 2). Eighteen patients (89%) had received prior therapy (median, 2 regimens; range, 0 to 6 regimens); 2 had received a prior allogeneic stem cell transplant. A total of 42 cycles were administered with a median of 1 cycle (range, 0 to 8 cycles); 10 patients (37%) received 2 or more cycles of therapy. The most common non-hematological toxicities of any grade, regardless of association with drug, were fatigue, diarrhea, dysphonia, dyspnea, hypertension, constipation, and abdominal pain. Two of 6 patients treated at the 375 mg/day dose level developed dose-limiting toxicities (DLT), consisting of grade 3 fatigue. The next dose cohort was therefore decreased to 325 mg/day; one patient on this dose level developed a DLT with grade 3 fatigue. However, as 2 additional patients subsequently developed grade 2 or 3 fatigue (during cycles 1 and 2, respectively), the 325 mg/day dose was not believed to be tolerable for chronic administration in this patient population. The next dose cohort was therefore decreased to 275 mg/day; 2 patients subsequently developed DLTs consisting of grade 3 typhilitis and grade 3 syncope, respectively. Overall, no patient experienced grade 4 toxicities or death from ENMD-2076. Of the 20 evaluable patients, one patient achieved a CRi (transfusion-independent with platelets <100 x 10^9/L), three experienced a morphologic leukemia-free state (MLFS) with a major HI-P and/or HI-E, and 4 other patients had an 11%, 14%, 23%, and 65% reduction in marrow blast count, respectively. *Ex vivo* studies showed pre-incubation with ENMD-2076 in 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 stimulation of 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. The recommended phase 2 dose (RPTD) in this patient population is 225 mg/day.

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

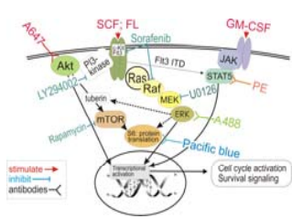
- Relapsed or refractory AML, acute lymphocytic leukemia (ALL), or chronic myeloid leukemia in blast crisis (CML-BC), chronic myelomonocytic leukemia (CMML), chronic lymphocytic leukemia (CLL), agnogenic myeloid metaplasia (AMM), lymphoma or myeloma
- Patients with untreated AML who refuse induction 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 SCF and FLT3 ligands 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) Exploratory testing for Aurora kinase inhibition by DNA content analysis of circulating blast cells

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=27)

Characteristic	All (n=27)
Age, y, median (range)	**69 (43-84)**
Gender	**7 F : 20 M**
ECOG status, median (range)	**1 (0-2)**
Acute myeloid leukemia	26 (96%)
De novo	19 (73%)
Therapy/Secondary	7 (27%)
Chronic Myelomonocytic Leukemia	1 (4%)
Disease duration, mo, median (range)	**6.6 (0.8-76.8)**
Flt3-ITD mutation status	
Negative	11 (41%)
Positive	3 (11%)
Unknown	13 (48%)
No. prior therapies, median (range)	**2 (0-6)**
None	3 (11%)
1 to 6 regimens*	24 (89%)

* allogeneic SCT (n=2)

Table 2. Most common non-hematological toxicities, regardless of attribution, for Cycle 1 (n=27)

Adverse Effect	Grade 1/2 No. (%)	Grade 3/4 No. (%)	Total No. (%)
Fatigue	16 (59)	4 (15)	20 (74)
Diarrhea	10 (37)	0	10 (37)
Hypertension	7 (26)	2 (7)	9 (33)
Dysphonia	8 (30)	0	8 (30)
Febrile neutropenia	0	7 (26)	7 (26)
Constipation	6 (22)	1 (4)	7 (26)
Dizziness	6 (22)	0	6 (22)
Hypomagnesemia	6 (22)	0	6 (22)
Nausea	5 (18)	1 (4)	6 (22)
Cough	5 (18)	0	5 (18)
Abdominal pain	4 (15)	1 (4)	5 (18)
Dyspnea	5 (18)	0	5 (18)
Confusional state	4 (15)	1 (4)	5 (18)

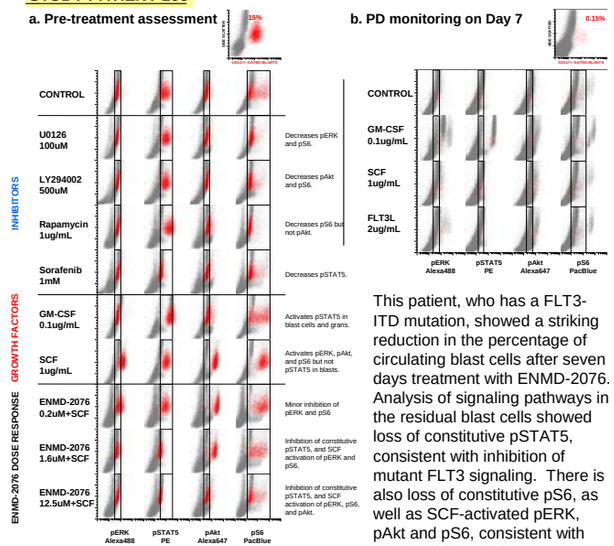
- 2 of 6 patients treated at dose level 375 mg/day developed DLT (grade 3 fatigue); therefore, the dose was reduced to 325 mg/day.
- 1 of 9 patients treated at dose level 325 mg/day developed DLT (grade 3 fatigue); 2 additional patients subsequently developed grade 2 or 3 fatigue (during cycles 1 and 2, respectively), therefore, the dose was reduced to 275 mg/day.
- 2 of 5 patients treated at dose level 275 mg/day developed DLT (grade 3 typhilitis & grade 3 syncope).

Table 3. Overall responses by IWG criteria (2003) (n=20)

	All (n=20)
$CR_{incomplete}$	1
Morphological LFS with major HI-P &/or HI-E	3
Overall Response	**4 (20%)**

- 4 other patients (Patients #119, 112, 126, & 120) had an 11%, 14%, 23%, and 65% reduction in marrow blast count, respectively
- Median time on study was 1.2 months (range 0.2-6.9 months) with 5 patients receiving more than 5 months of therapy

STUDY PATIENT 106



This patient, who has a FLT3-ITD mutation, showed a striking reduction in the percentage of circulating blast cells after seven 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, as well as SCF-activated pERK, pAkt and pS6, consistent with c-Kit inhibition during treatment with ENMD-2076.

Summary of pharmacodynamic monitoring

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. Patient 120 had a similar profile to Patient 106 and had a 65% reduction in blasts, 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.
- The recommended phase 2 dose (RPTD) of ENMD-2076 in this patient population is 225 mg orally daily.
- 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].

DISCLOSURES: C. F. Sidor, G. Fletcher, J. Arnott and M. Bray are employees of EntreMed, Inc.