Clinical Safety and Efficacy of the Aurora and Angiogenic Kinase Inhibitor ENMD-2076 in Previously Treated, Locally Advanced or Metastatic Triple-Negative Breast Cancer

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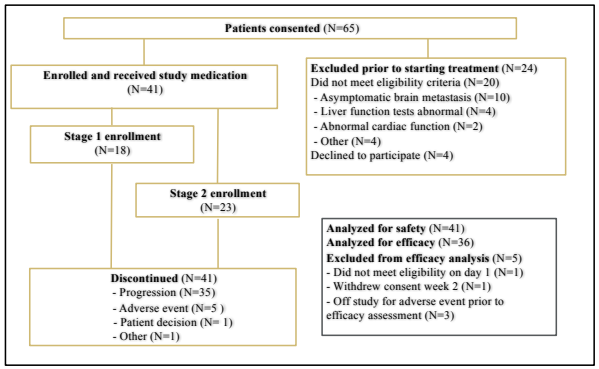
ABSTRACT

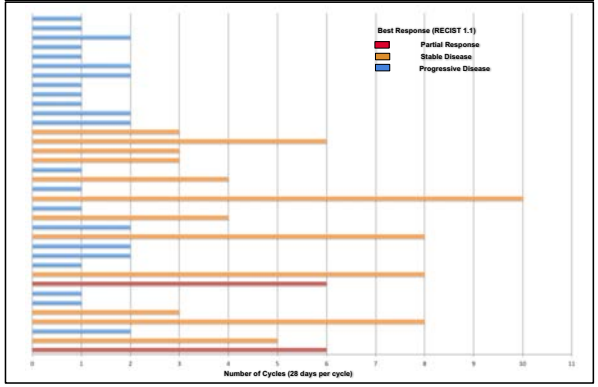
Background: Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype defined by the lack of expression of the estrogen and progesterone receptors and lack of HER2 over-expression. ENMD-2076 is an orally bioavailable small molecule inhibitor of Aurora and angiogenic kinases with pro-apoptotic and antiproliferative activity in preclinical models of TNBC.
Methods: This two institution, single-arm, two-stage, phase II clinical trial enrolled patients with locally advanced or metastatic TNBC refractory to 1-3 prior lines of chemotherapy in the advanced setting. Patients had ECOG PS ≤ 1, measureable disease by RECIST 1.1 and no evidence of brain metastasis. Patients were treated with ENMD-2076 250 mg PO daily with continuous dosing in 4-week cycles until disease progression or unacceptable toxicity occurred. The primary end point was 6-month clinical benefit rate (6-CBR) and secondary endpoints included time to progression (TTP), PK profile, safety and biologic correlatives in archival and fresh serial tumor biopsies in a subset of patients.
Results: Between July 2012 and October 2016, 41 patients were enrolled (median age 54; range 30-73; female 40; male 1). Patients received a mean 1.7 prior lines of chemotherapy for locally advanced unresectable or metastatic disease and 80.5% received prior neoadjuvant or adjuvant chemotherapy (N=33). Thirty-six patients were evaluable per protocol for the primary efficacy analysis. Five patients (12.2%) were not included in the efficacy analysis due to: adverse events (AE) leading to discontinuation prior to objective efficacy assessment (N=3), not meeting eligibility criteria on day 1 (N=1) and withdraw of consent in cycle 1 (N=1). The study proceeded to the second stage of enrollment based on observing three 6-CBR events in Stage 1 (N=18 patients). The 6-CBR in the overall trial was 16.7% (95% exact CI: 6%-32.8%; 2 patients with PR and 4 patients with SD > 6 mos). The 4-CBR was 27.8% (95% exact CI: 14%-45.2%). The median duration of response or clinical benefit in these patients was 32 weeks (8 cycles). Dose reduction occurred in 8 patients (20%) for fatigue, hypertension and proteinuria. The most common grade 3 treatment-related adverse events were hypertension (37.5%) and fatigue (10%). One patient experienced grade 4 hypertension. Analysis of serial tumor biopsies prior to and following 2 weeks of ENMD-2076 (N=8 patients), demonstrated a treatment-induced decrease in cellular proliferation (Ki-67) and microvessel density (CD34) as assessed by IHC. Immunofluorescence performed on a subset of samples demonstrated an increase in p53-family member expression following treatment, consistent with changes observed in preclinical TNBC patient-derived tumor xenograft models.
Conclusions: ENMD-2076 has durable clinical activity in a subset of patients with pretreated, advanced or metastatic triple-negative breast cancer. Predictive biomarker development using archival and fresh tumor tissue is underway. Exploration of lower doses of ENMD-2076 in future clinical trials may improve tolerability.

PATIENT CHARACTERISTICS

Characteristic	Number of Patients (N=41)
Age, years Median (Range)	 54 (30-73)
Sex Male Female	 1 (2.4%) 40 (97.6%)
Race White Black or African American Unknown	 33 (80.5%) 5 (12.2%) 3 (7.3%)
ECOG Performance Status 0 1	 19 (46.3%) 22 (53.7%)
Prior Lines of Systemic Therapy Metastatic Disease Mean 1 2 3	 1.7 21 (51.2%) 10 (24.4%) 10 (24.4%)
Prior Neoadjuvant or Adjuvant Chemotherapy Yes No	 33 (80.5%) 8 (19.5%)
BRCA1/2 Germline Mutation Mutated Wildtype Unknown	 4 (9.8%) 19 (46.3%) 18 (43.9%)
Sites of Metastasis Lung Lymph Nodes Liver Bone Chest Wall Other	 15 (24.4%) 20 (48.8%) 15 (24.4%) 16 (39.0%) 8 (19.5%) 6 (14.6%)



DURATION ON STUDY



EFFICACY

Efficacy Response	Number of Patients (N=36)
Complete Response	0 (0%)
Partial Response	2 (5.6%)
Stable Disease	14 (38.9%)
Progressive Disease	20 (55.6%)
4 mos Clinical Benefit Rate	10 (27.8%)
6 mos Clinical Benefit Rate	6 (16.7%)

SAFETY PROFILE

Treatment-related adverse events occurring in ≥ 10% of patients

	Number of Patients (N=41)	
Adverse Event	**All Grades**	**Grade ≥ 3**
Hypertension	27 (66%)	16 (39%)
Fatigue	22 (54%)	4 (10%)
Diarrhea	22 (54%)	1 (2%)
Nausea	20 (49%)	0 (0%)
Constipation	10 (24%)	0 (0%)
Headaches	10 (24%)	0 (0%)
Vomiting	9 (22%)	1 (2%)
Mucositis	6 (15%)	1 (2%)
Proteinuria	6 (15%)	3 (7%)
Dysgeusia	5 (12%)	0 (0%)
GERD	5 (12%)	0 (0%)
Anorexia	4 (10%)	0 (0%)

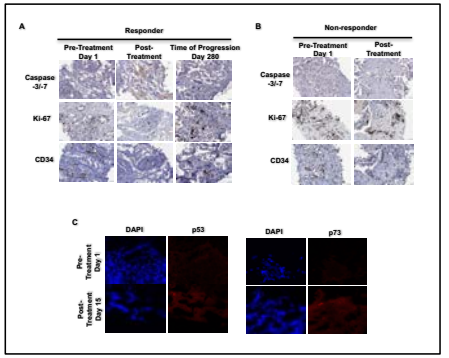


Figure 1. Effects of ENMD-2076 on pharmacodynamic markers in serial tumor biopsies. A. Responder. Caspase -3/-7, Ki-67 and CD34 on serial tumor biopsies to assess apoptosis, proliferation and microvessel density, respectively, in a patient responding to ENMD-2076 treatment with prolonged stable disease for 10 cycles. Biopsies were obtained prior to treatment, 15 days after treatment, and at the time of disease progression day 280. FFPE tissue sections were stained with the indicated antibodies and and representative images were taken at 20X magnification. Note an increase in caspase -3/-7 and a decrease in Ki-67 and CD34 in the post-treatment biopsy. At the time of disease progression, these changes were reversed. **B. Non-responder**. Stains were performed as in panel A. Note there is no decrease in proliferation or increase in apoptosis in the non-responder following ENMD-2076 treatment. **C. Immunofluorescence analysis** of tumor biopsies for DAPI, p53, and p73 in a patient who had stable disease by RECIST 1.1 after 2 cycles of treatment and then progressed after cycle 3. Patient has a p53 mutation R273S. Note an increase in p53 and p73 following treatment which is consistent with preclinical findings in PDX models. IF images were acquired using confocal microscopy at 60X magnification.

p53 MUTATION STATUS

p53 Mutations in Archival Tissue Samples	N (%)
Deleterious p53 mutation (any sample)	N=36
Yes	19 (52.7%)
No (wildtype)	13 (36.1%)
Unknown	4 (11.1%)
Response at 2 mos in pts with p53 mutation	N=19
SD or PR at 2 mos	9 (47.4%)
PD at or before 2 mos	10 (52.6%)
Response at 2 mos in pts with p53 WT	N=13
SD or PR at 2 mos	5 (38.5%)
PD at or before 2 mos	8 (61.5%)

Sanger sequencing of p53 exons 4-8 and 10

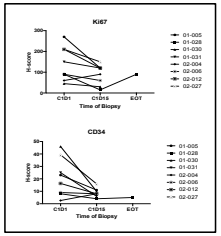


Figure 2. Changes in Ki67 and CD34 (microvessel density) in serial tumor biopsies. Baseline and D15 samples were available in 8 patients. An additional sample was obtained in 1 patients at the time of progression following prolonged stable disease. Ki67 and CD34 were assessed using IHC.

CONCLUSIONS

- ENMD-2076 has durable clinical activity in a subset of patients with pretreated, advanced or metastatic triple-negative breast cancer.
- A mechanism-based adverse event profile was observed with hypertension and gi toxicity.
- Deleterious p53 mutations were detected in approximately half of patients in archival tissue (52.7%).
- An increase in apoptosis and a decrease in proliferation and microvessel density was observed in serial tumor biopsies in patients responding to treatment. An increase in p53 and p73 was observed following treatment consistent with our findings previously reported in p53-mutated TNBC PDX models (Ionkina et al. MCT 2017).
- Predictive biomarker development using archival and fresh tumor tissue is underway.
- Exploration of lower doses of ENMD-2076 in future clinical trials may improve tolerability.

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