

THE ANGIOKINASE INHIBITOR, ENMD-2076, IN COMBINATION WITH CISPLATIN INDUCES TUMOR REGRESSION IN A MULTIDRUG RESISTANT, TRIPLE-NEGATIVE HUMAN BREAST CARCINOMA MODEL

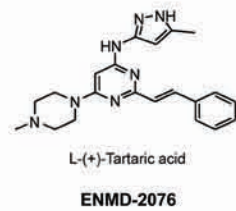
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ABSTRACT

ENMD-2076 is an orally available small molecule with Aurora A and angiokinase inhibitory activity associated with VEGFRs, FGFRs and PDGFRs. ENMD-2076 has demonstrated substantial, dose-dependent activity in multiple xenograft models and is currently undergoing Phase I clinical studies in advanced solid tumors and multiple myeloma. To support the clinical development of ENMD-2076, we have begun to evaluate combination approaches. These studies were designed to determine the antitumor activity of ENMD-2076 in combination with cisplatin (CDDP) using the orthotopically implanted, triple-negative human breast carcinoma, MDA-MB-231 (MDA-MB-231^{MDR1}), or its multidrug resistance variant, MDA-MB-231^{MDR1}. Treatments were initiated at a mean tumor volume of ~400-500 mm³ and consisted of ENMD-2076 alone (200, 100 or 50 mg/kg/day, po), CDDP alone (6, 3, or 1 mg/kg/week, ip), or combinations of ENMD-2076 with CDDP. All treatments were well tolerated by tumor bearing mice. The results demonstrate a substantial combination antitumor effect in both tumor models. For example, in the MDA-MB-231^{MDR1} model, treatment with ENMD-2076 (100 mg/kg/day) and CDDP (6 mg/kg/week) resulted in tumor regression in 12/18 mice, compared to 2/19 mice in each group treated with either agent alone. Immunohistochemical analyses of tumor tissue indicated that combined treatment resulted in enhanced antiangiogenic, antiproliferative, and proapoptotic activity above that of either agent administered alone. Collectively, these results suggest that the clinical evaluation of ENMD-2076 in combination with CDDP is warranted.

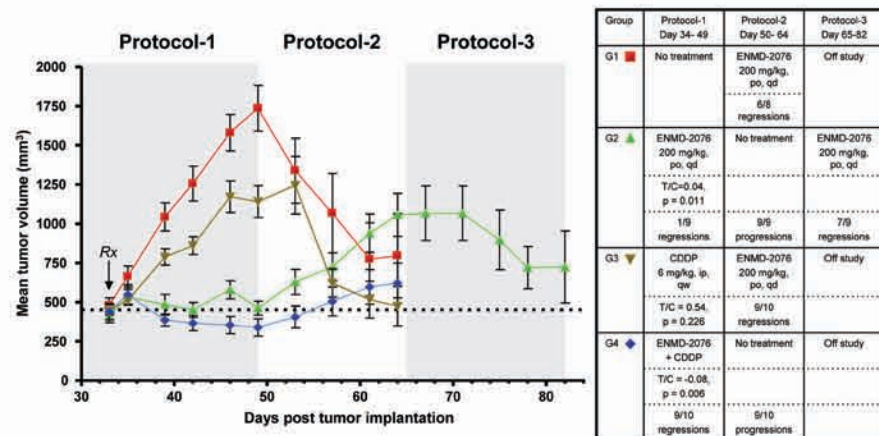
INTRODUCTION AND RESULTS

Figure 1 and Table 1. ENMD-2076 is an Aurora and Angiogenic Kinase Inhibitor



Kinase	Recombinant Protein IC ₅₀ (nM)	Cellular IC ₅₀ (nM)
AurA	14	130
AurB	310	2400
KDR/VEGFR2	36	80
Flt4/VEGFR3	16	nd
FGFR1	93	600
FGFR2	71	nd
PDGFR α	56	1000-5000

Figure 2 and Table 2. Antitumor Activity of ENMD-2076 Alone or in Combination with CDDP on Orthotopic, Triple-Negative MDA-MB-231^{MDR1} Tumors in C.B-17 SCID Mice

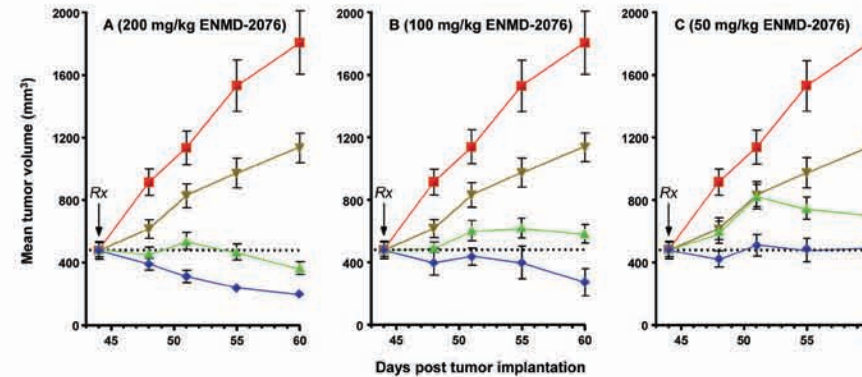


Methods and Results
Protocol-1: Beginning 33 days post orthotopic implantation of MDA-MB-231^{MDR1} cells when mean tumor volume was ~450 mm³, mice were randomized into four treatment arms (n=8-10/arm) to receive: G1 = no treatment; G2 = ENMD-2076 (200 mg/kg, po, qdx16); G3 = CDDP (6 mg/kg, ip, qwx3); and G4 = ENMD-2076 + CDDP. No treatment related morbidity was observed. Tumor bearing mice treated with CDDP or ENMD-2076 had T/Cs of 0.54 and 0.04, respectively, with no regressions in the CDDP alone arm and 1/9 tumors showing regression in the ENMD-2076 alone arm. Mice treated with the combination of ENMD-2076 and CDDP had a T/C of 0.08, with 9/10 mice showing clear evidence of tumor regression.
Protocol-2: At the conclusion of protocol-1 (49 days post tumor implantation), mice from G1 and G3 began treatment with ENMD-2076 (200 mg/kg, po, qdx14), and mice from G2 and G4 had treatment halted. Irrespective of tumor size or prior treatment with CDDP, mice treated with ENMD-2076 demonstrated pronounced tumor responses with 5/8 and 9/10 mice showing tumor regression in G1 and G3, respectively. Tumor regrew in G2 (9/9 progression) and G4 (9/10 progression) when treatment was stopped.
Protocol-3: Following the end of protocol-2 (65 days post tumor implantation), mice from G2 had treatment reinitiated with ENMD-2076 (200 mg/kg, po, qdx17). Mice from G1, G3 and G4 were removed from study. Reinitiation of treatment with ENMD-2076 resulted in tumor regressions in 7/9 mice.

Summary (Figure 2)

- Treatment of MDA-MB-231^{MDR1} xenografts with ENMD-2076 resulted in tumor stasis or regression.
- In this tumor model, a maximally tolerated dose of CDDP inhibited tumor growth by approximately 50%.
- Combined treatment with ENMD-2076 and CDDP resulted in a synergistic antitumor effect as assessed by overall regression compared to either agent alone.

Figure 3 and Table 3. Dose-dependent Antitumor Activity of ENMD-2076 Alone or in Combination with CDDP on Multidrug Resistant, Triple-Negative MDA-MB-231^{MDR1} Tumors in C.B-17 SCID Mice



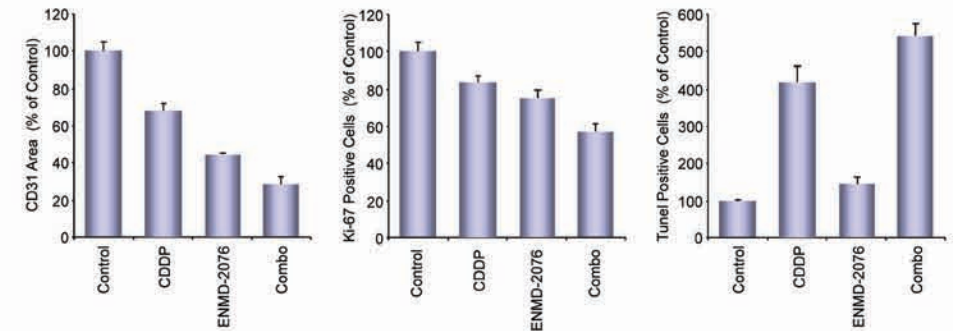
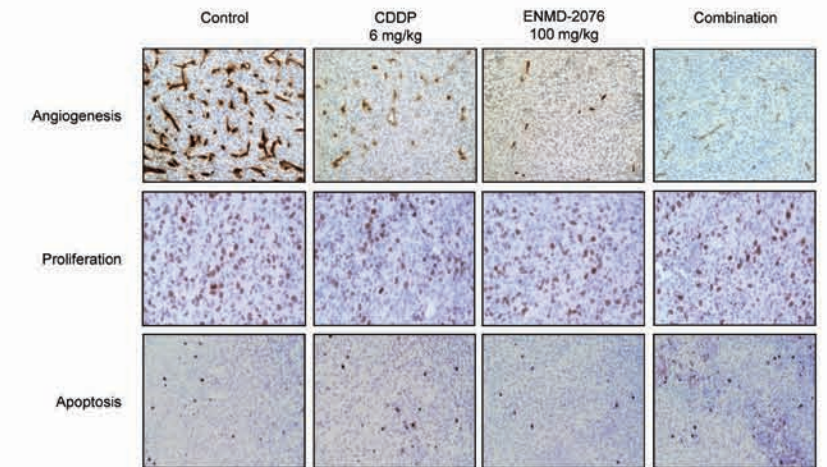
Group	G1	G2	G3	G4
A	No treatment	ENMD-2076 200 mg/kg, po, qd	CDDP 6 mg/kg, ip, qw	ENMD-2076 + CDDP
B	No treatment	ENMD-2076 100 mg/kg, po, qd	CDDP 6 mg/kg, ip, qw	ENMD-2076 + CDDP
C	No treatment	ENMD-2076 50 mg/kg, po, qd	CDDP 6 mg/kg, ip, qw	ENMD-2076 + CDDP

Methods and Results
 Beginning 44 days post orthotopic implantation of MDA-MB-231^{MDR1} cells when mean tumor volume was ~500 mm³, mice were randomized into four treatment arms (n=8-10/arm) to receive: G1 = no treatment; G2 = ENMD-2076 (A = 200 mg/kg, po, qdx16; B = 100 mg/kg, po, qdx16; C = 50 mg/kg, po, qdx16); G3 = CDDP (6 mg/kg, ip, qwx3); and G4 = ENMD-2076 + CDDP. ENMD-2076 was orally administered, daily for 16 consecutive days. No treatment related morbidity was observed. Tumor bearing mice treated with ENMD-2076 (200, 100, 50 mg/kg) or CDDP had T/Cs of -0.09, 0.07, 0.22, and 0.36, respectively, with 7/9 and 1/9 mice showing regression in the ENMD-2076 alone arm (200 and 100 mg/kg, respectively). Mice treated with the combination of ENMD-2076 (200, 100, and 50 mg/kg) and CDDP had T/Cs of -0.22, -0.13, and 0.02, with 9/9, 7/8, and 2/8 mice showing regression, respectively.

Summary (Figure 3 and Table 3)

- Treatment of multidrug resistant, triple-negative MDA-MB-231 xenografts with ENMD-2076 resulted in dose-dependent antitumor activity as measured by tumor regression or growth inhibition.
- In the MDA-MB-231^{MDR1} tumor model, a maximally tolerated dose of CDDP inhibited tumor growth by approximately 64%.
- Combined treatment with ENMD-2076 and CDDP resulted in synergistic antitumor effects. For example, treatment with ENMD-2076 (100 mg/kg/day) and CDDP (6 mg/kg/week) resulted in tumor regression in 7/8 mice, compared to 1/9 and 0/9 mice treated with ENMD-2076 or CDDP alone, respectively.

Figure 4. Impact of ENMD-2076 Alone or in Combination with CDDP on Angiogenesis, Proliferation and Apoptosis of Triple-Negative MDA-MB-231^{MDR1} Tumors in C.B-17 SCID Mice



Methods and Results
 At study termination (day 60, Figure 3), tumors were collected from mice treated with control vehicle, CDDP (6 mg/kg, ip, qwx3), ENMD-2076 (100 mg/kg, po, qdx16), or ENMD-2076 + CDDP and processed for CD31, Ki-67, or Tunel staining. As compared to control, vascular area was decreased ~40, 60, and 80% in tumors from mice treated with CDDP, ENMD-2076, or the combination, respectively. Tumor proliferative status was decreased by ~20% in mice treated with CDDP or ENMD-2076 and by ~40% with the combination. Incidence of apoptotic cells was increased by ~4, 1.5, and 5.5-fold in tumors from mice treated with CDDP, ENMD-2076, or the combination, respectively.

Summary (Figure 4)

- Enhanced antitumor activity is observed with the combination of ENMD-2076 and CDDP, over either agent alone.
- The antitumor activity correlates with biomarkers of angiogenesis, proliferation and apoptosis.

CONCLUSIONS

ENMD-2076, an Aurora A and angiokinase inhibitor, synergizes with CDDP for antitumor activity in a multidrug resistant, triple-negative breast adenocarcinoma model. Triple-negative breast cancer largely represents a subtype of breast tumors clinically characterized as more aggressive and less responsive to standard treatment and associated poorer overall patient prognosis. Multiple approaches to improved care of triple-negative breast cancer, including DNA-damaging agents such as platinum, are under investigation. The results reported here provide a rationale for the clinical evaluation of ENMD-2076 in combination with CDDP in this disease.

