

# ENMD-2076 Exerts Antiangiogenic and Antiproliferative Activity Against Human Colorectal Cancer (CRC) Xenograft Models

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## Abstract

**Background:** ENMD-2076 is a novel, small molecule kinase inhibitor with activity against Aurora A as well as multiple tyrosine kinases linked to cancer, including VEGFR2, cKit and PDGFR $\alpha$ . As a result, ENMD-2076 exerts its effects through multiple mechanisms of action, including antiproliferative activity and the inhibition of angiogenesis. The goal of the current study was to test the efficacy and potential toxicity of ENMD-2076 in a mouse xenograft model of CRC.

**Methods and Results:** Athymic nude mice were injected subcutaneously in the left flank with  $2 \times 10^6$  HT29 CRC cells. When tumors reached a volume of 100 mm<sup>3</sup>, mice were randomized into three groups: 1) vehicle, 2) ENMD-2076 (100 mg/kg), or 3) ENMD-2076 (200 mg/kg); n=5 per group. Vehicle or drug was administered p.o., q.d, for 28 days by oral gavage. ENMD-2076 was well-tolerated, with no apparent toxicity and no significant weight loss over the course of the study, at either dose. Tumor volume measurements, taken every 3 days, revealed initial stasis in tumor growth in mice treated with either dose of ENMD-2076 and tumor regression at the 200 mg/kg dose beginning on day 18 and continuing to the end of the study. Tumors in the mice treated with the 200 mg/kg dose also displayed significant blanching, indicating a loss of tumor vascularity. To further quantify the effects of ENMD-2076 on tumor angiogenesis, gadolinium (Gd) based dynamic-contrast enhanced magnetic resonance imaging (DCE-MRI) was performed. Three animals per group underwent DCE-MRI scans at baseline and days 7 and 28 after the initiation of treatment. The initial area under the Gd-curve (IAUC) of the tumor, calculated for the first 90 seconds post Gd injection, were significantly lower versus control for both the 100 and 200 mg/kg treatment groups on day 28 (p<0.05) indicating decreased vascular perfusion. Finally, at study end, the tumors were resected and histologically examined. Tumors from the mice treated with 200 mg/kg ENMD-2076 showed significant areas of necrosis compared to controls. IHC analysis for Ki-67 demonstrated a dramatic decrease in the number of proliferating cells in the tumors from mice treated with 200 mg/kg ENMD-2076 compared to vehicle controls.

**Conclusions:** The results of this study indicate that ENMD-2076 has antitumor effects on an HT29 CRC xenograft model. We observed tumor stasis and regression in mice treated with 200 mg/kg ENMD-2076. DCE-MRI and post-study histological examination suggests that these antitumor effects are exerted through a combination of antiangiogenic and antiproliferative actions. These preclinical studies provide evidence that ENMD-2076 may be an effective therapy option for clinical treatment of CRC.

**UPDATE:** Further studies were conducted to determine the effects of ENMD-2076 at the dose of 100 mg/kg on HT29 xenografts with an initial volume of 300 mm<sup>3</sup>. In addition to DCE-MRI, we performed positron emission tomography (PET) analysis at baseline, Day 3 and Day 21 of treatment with vehicle or ENMD-2076. We observed significant reductions in <sup>18</sup>FDG uptake at Day 3 and Day 21 in mice treated with ENMD-2076, indicating both early and sustained effects on tumor metabolic activity.

## Materials and Methods

**Animals and Xenograft Models:** Female athymic nude mice were injected subcutaneously in the left flank with  $2.5 \times 10^6$  HT29 colorectal cancer cells. Tumors were measured every three days by caliper until desired volume was achieved. Animals were then randomized into their respective groups.

**Drug Preparation and Dosing:** ENMD-2076 was dissolved in sterile water and sonicated for 5 min, to achieve complete solubility. Drug was administered via oral gavage.

**Positron Emission Tomography (PET):** Animals were maintained un-anesthetized on heated water pad for approximately one hour to allow for <sup>18</sup>FDG uptake in tumors. Under isoflurane anesthesia, mice were placed on a heating pad (m2m imaging) and a 10 minute emission was acquired with a PET scanner (Inveon, Siemens Medical). Images were analyzed with AsiproVM. Regions of interest (ROIs) were drawn with the trace command around the tumors on axial slices. The total activity of all tumor slices were summed. Activity was divided by the time-corrected dose delivered (time corrected dose = dose injected  $\times$  exp (-0.006317 $\times$ t), where t is the time between the injection and scan time) and is presented as the percentage of the respective tumors baseline scan.

**Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI):** Animals were anesthetized with 60 mg/kg xylazine, 10 mg/kg ketamine and a tail vein catheter was inserted immediately prior to imaging. After 1 min precontrast images, 0.1 mmol/kg Gd-DTPA contrast agent (Magnevist, Berlex Schering AG) was given i.v. during fast T1-MRI scans for another 14 min. Proton density and T1-weighted images were acquired on a Bruker Pharma Scan animal scanner at 4.7 T. All data were processed using Bruker ParaVision Software.

## Results

Figure 1: HT29 Colorectal Tumor Xenograft Growth Curves

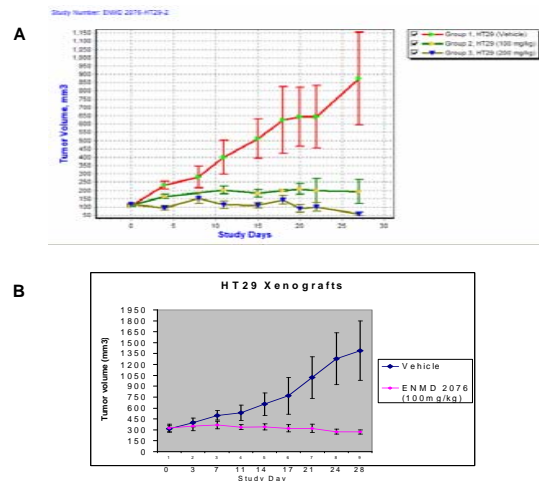


Figure 1: HT29 Xenograft growth curves from two separate experiments. In panel A, tumors on flanks of female nude mice reached an average volume of 100 mm<sup>3</sup> before randomization and dosing with vehicle or ENMD-2076 at 100 mg/kg or 200 mg/kg (n=5 per group). In panel B, tumors reached an average volume of 300 mm<sup>3</sup> before randomization and dosing with vehicle or ENMD-2076 at 100 mg/kg (n=12 per group).

Figure 2: HT29 Tumor Xenografts 12 and 28 Days Post-treatment With ENMD-2076

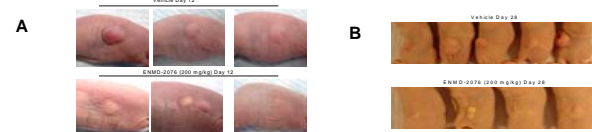


Figure 2: Nude mice bearing HT29 xenografts (A) 12 days and (B) 28 after treatment with vehicle or ENMD-2076 at 200 mg/kg. Note progressive "blanching" of tumors in the treated group, indicating a loss of tumor vascularity.

Figure 3: Excised HT29 Xenograft Tumors Treated With ENMD-2076 Show Reduced Vascularity and Proliferation As Measured by Ki-67 Staining

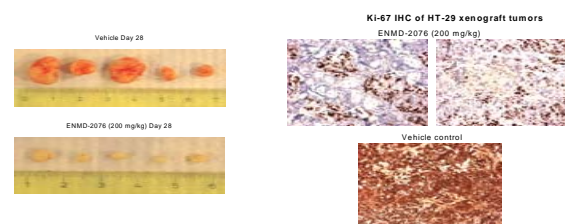


Figure 4: Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI) Analysis of HT29 Xenograft Tumors Treated With ENMD-2076

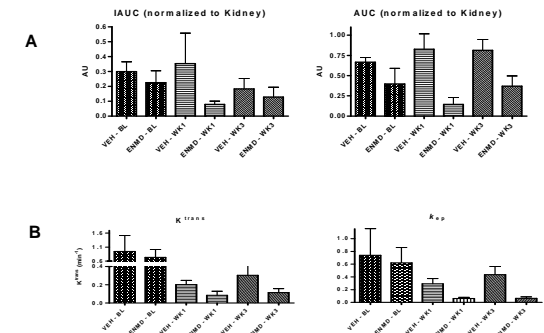


Figure 4: DCE-MRI analysis of HT29 xenograft tumors. Gadolinium (Gd)-based DCE-MRI scans were performed at baseline (BL), one week (WK1) and three weeks (WK3) post-treatment with vehicle (VEH) or ENMD-2076 (100 mg/kg). A) Initial Gd uptake (IAUC), a measure of tumor perfusion, was calculated for the first 90 seconds post-injection, and is depicted as initial area under the curve (AUC). The subsequent 14 min Gd uptake (AUC) was used to assess tumor perfusion and permeability. Both IAUC and AUC values were inhibited by treatment with ENMD-2076. B)  $K^{trans}$  and  $K^{ip}$  data was calculated using the 2-compartment Tofts model (Tofts, et al, J Mag Reson Imaging 10:223, 1999).  $K^{trans}$ , the volume transfer constant of contrast agent between blood plasma and the tumor extravascular extracellular space, was decreased >50% compared to vehicle controls 1 wk and 3 wk post-treatment with ENMD-2076, indicating a decrease tumor vascular permeability. Similarly,  $K^{ip}$ , the reflux coefficient which assesses transfer of contrast agent from the tumor back to the blood was inhibited ~70% after ENMD-2076 treatment.

Figure 5: Positron Emission Tomography (PET) Analysis of HT29 Xenograft Tumors Treated With ENMD-2076

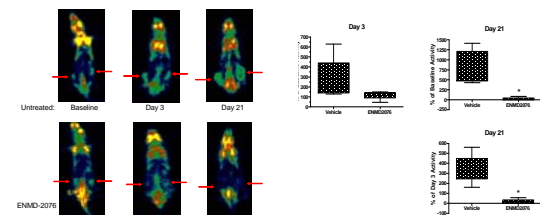


Figure 5: PET Analysis. PET was performed on animals prior to and 3 and 21 days post-treatment. <sup>18</sup>FDG was administered to animals by tail vein injection. Our data shows that as early as 3 days post-treatment differences in tumor uptake of <sup>18</sup>FDG can be measured. By day 21 post-treatment, tumors were barely detectable or completely undetectable by PET.

## Conclusions

- ENMD-2076 is a novel, small molecule kinase inhibitor with activity against Aurora A as well as multiple tyrosine kinases linked to cancer, including VEGFR2, cKit and PDGFR $\alpha$ .
- ENMD-2076 demonstrated strong anti-tumor effects on an HT29 CRC xenograft model with tumor stasis and regression observed in mice treated with either 100 or 200 mg/kg ENMD-2076.
- DCE-MRI and post-study histological examination suggests that these antitumor effects are exerted through a combination of antiangiogenic and antiproliferative actions.
- PET analysis demonstrated an early and sustained decrease in tumor metabolic activity at Day 3 and Day 21 post-treatment with ENMD-2076 (100mg/kg).
- These preclinical studies provide evidence that ENMD-2076 may be an effective therapy option for clinical treatment of CRC.