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 PDGFRα. 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: Aithylic nude mice were injected subcutaneously in the left flank with 2×106 HT29 CRC cells. When tumors reached a volume of 100 mm3, mice were randomized into three groups: 1) vehicle, 2) ENMD-2076 (100 mg/kg), or 3) ENMD-2076 (200 mg/kg). Vehicle or drug was administered p.o., i.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. 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 tumors, 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 (<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.

Materials and Methods

Animals and Xenograft Models: Female athylic nude mice were injected subcutaneously in the left flank with 2×106 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 Dosering: 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 an-anesthetized on heated water pad for approximately one hour to allow for 18FDG 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 AriaPro/VM. 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 x exp (- λ t); where λ 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 ml/kg Gd-DPTA 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 7 T. All data were processed using Bruker Paravision Software.

Results

Figure 1: HT29 Colorectal Tumor Xenograft Growth Curves

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

Figure 3: Excised HT29 Xenograft Tumors Treated With ENMD-2076 Show Reduced Vascularization 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 5: Positron Emission Tomography (PET) Analysis of HT29 Xenograft Tumors Treated With ENMD-2076

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 PDGFRα.

•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 (100 mg/kg).

•These preclinical studies provide evidence that ENMD-2076 may be an effective therapy option for clinical treatment of CRC.