

2ME2 and ENMD-1198: Microtubule Targeting Agents That Downregulate HIF and Inhibit Angiogenesis

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2-Methoxyestradiol (2ME2) and the 2ME2 analog, ENMD-1198 (2-methoxyestra-1, 3, 5, (10) 16-tetraene-3-carboxamide) have significant *in vitro* and *in vivo* antitumor and antiangiogenic activities. 2ME2 and ENMD-1198 exhibit antiproliferative activity against a wide range of tumor and endothelial cell types *in vitro*, destabilize microtubules and inhibit hypoxia-inducible factor (HIF)-1 α activity. Microtubule disruption assessed by immunofluorescence correlated with decreased protein levels of HIF-1 α , HIF-2 α , NF κ B, and Stat3. *In vivo* tumor effects subsequent to oral dosing were assessed by comparing vehicle and ENMD-1198 treated animals for survival or tumor volume, immunohistochemical (IHC) staining for apoptosis (TUNEL), proliferation (Ki-67), angiogenesis (CD-31), HIF-1 α , carbonic anhydrase IX (CA IX). Circulating human vascular endothelial growth factor (hVEGF) and interleukin-6 (hIL-6) levels were assessed. ENMD-1198 resulted in significantly improved median survival time in a Lewis lung carcinoma metastatic model (200 mg/kg, Cox Proportional Hazard, $P < 0.05$); reduced tumor volumes in both an MDA-MB-231 orthotopic breast tumor model (200, 300 and 400 mg/kg); and in an HT-29 colon carcinoma model (100, 200 and 400 mg/kg) (ANOVA, $p < 0.05$). Decreased circulating levels of hVEGF and hIL-6 were observed in the breast cancer model (300 and 400 mg/kg) (ANOVA, $P < 0.05$). IHC analyses indicated increased staining area of TUNEL-positive cells and decreased staining area of HIF-1 α , active NF κ B, active Stat3, and Ki-67 compared to vehicle in MDA-MB-231 tumors from mice treated with ENMD-1198 ($P < 0.05$). Decreases in HIF-1 α , CD31 and hVEGF levels *in vivo* are consistent with ENMD-1198 inhibiting angiogenesis. ENMD-1198 is entering Phase 1 clinical studies for use as an anticancer agent, given the robust antitumor activity and the inhibition of transcription factors with key roles in tumorigenesis and angiogenesis.